ASSOCIATION OF SERUM NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL) LEVELS WITH PREECLAMPSIA

Dissertation submitted in

Partial fulfillment of the regulations required for the award of

M.D. DEGREE

BIOCHEMISTRY – BRANCH XIII

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

PSG INSTITUTE OF MEDICAL SCIENCES AND RESEARCH

COIMBATORE

APRIL – 2017

CERTIFICATE

CERTIFICATE

This is to certify that the dissertation titled **"ASSOCIATION OF SERUM NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL) LEVELS WITH PREECLAMPSIA"** submitted by **Dr.M.Dhivya** is an original work done by her at PSG Institute of Medical Sciences and Research, Coimbatore. This work was done under the guidance of Dr.G.Jeyachandran,Professor&Head, Department of Biochemistry, PSG Institute of Medical Sciences and Research.

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Place : Coimbatore

Date :

DECLARATION

DECLARATION

I solemnly declare that this dissertation **"ASSOCIATION OF SERUM NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL) LEVELS WITH PREECLAMPSIA"** was written by me in the Department of Biochemistry, PSG Institute of Medical Sciences and Research, Coimbatore under the guidance of **Dr.G.Jeyachandran**, Professor and Head, Department of Biochemistry, PSG Institute of Medical Sciences and Research.

This dissertation is submitted to the Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment of the university regulations for the degree of M.D. Biochemistry – Branch XIII examinations to be held in April 2017.

Place: Coimbatore

Date: **Dr.M.DHIVYA**

ACKNOWLEDGEMENT

ACKNOWLEDGMENT

I thank The Almighty for His blessings.

*I express my sincere thanks to **Dr.S.Ramalingam**, Dean, PSG Institute of Medical Sciences and Research for granting me permission to conduct this study and utilize the facilities required for the study.*

*I would like to extend my heartfelt and sincere gratitude to my guide **Dr.G.Jeyachandran**, Professor and Head, Department of Biochemistry for his resolute motivation throughout my study period. It is his patience, trust on the student, immense knowledge that has made this study a feasible one.*

*I express my sincere thanks to **Dr.B.Gayathri**, Professor, Department of Biochemistry for her support in this endeavor. I wish to thank Professor **Dr.D.Vijaya** for her guidance.*

*It would have been difficult for me to have completed this study without **Dr.G.Sumitra**, Associate Professor of our Department, who with her expertise skills, knowledge, patience and empathetic nature has helped me to complete this study. I would like to thank **Dr.S.Kavitha,** Associate Professor of our Department for her help and motivation in my work. I would like to thank assistant professors of our department **Dr.A.S.Meenakshi Sundaram** and **Dr.R.Sujatha** for their help in my study. I wish to thank **Mrs.V.Aruna**, Lecturer for her help in my work.*

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I am extremely thankful to all my patients who consented to be a part of my study without whom the whole study would have been impossible.

ABBREVIATIONS

ABBREVIATIONS

ACOG-American Congress of Obstetricians and Gynaecologists

ALT-Alanine Transaminase

AKI-Acute Kidney Injury

AST-Aspartate Transaminase

BMI - Body mass index

BUN-Blood Urea Nitrogen

ELISA-Enzyme Linked ImmunoSorbent Assay

sENG-serum Endgolin

sFlt-serum Fms like tyrosine kinase

GFR-Glomerular Filtration Rate

Hb-Hemoglobin

HLA-Human Leukocyte Antigen

IL-18-Interleukin-18

IUGR-Intra Uterine Growth Retardation

LDH-Lactate Dehydrogenase

MMP-9-Matrix Metallo Proteinase-9

NGAL-Neutrophil Gelatinase Associated Lipocalin

NHBPEP-National High Blood Pressure Education Programme

NO-Nitric Oxide

PBS-Phosphate Buffered Saline

PCR-Protein Creatinine Ratio

PG-Prostaglandin

PRAKI-Pregnancy Related Acute Kidney Injury

ROS-Reactive Oxygen Species

SGA-Small for Gestational Age

SOMANZ-Society for Obstetric Medicine Australia and New Zealand

TMB-Tetra methyl Benzidine

TNF-Tumor Necrosis Factor

TXA_2-Thromboxane-A_2

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To
Dr M Dhivya
Postgraduate
Department of Biochemistry
PSG IMS & R
Coimbatore

Ref: Project No. 14/397

Date: January 19, 2015

Dear Dr Dhivya,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 05.12.2014 to conduct the research study entitled "*Association of serum NGAL (Neutrophil Gelatinase Associated Lipocalin) with pre-eclampsia*" during the IHEC review held on 19.12.2014.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol (Ver 1.1)
3. Informed consent form (Ver 1.1)
4. Data collection tool
5. Current CVs of Principal investigator, Co-investigators
6. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 19.12.2014 at College Council Room, PSG IMS & R between 2.00 pm am and 4.30 pm:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mrs Y Ashraf	MPT	Physiotherapy	Female	Yes	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Mr Gowpathy Velappan	BA., BL	Legal Advisor	Male	No	Yes
4	Mr P Karuppuchamy	M Phil in PSW	Social Scientist	Male	Yes	Yes
5	Mrs G Malarvizhi	M Sc	Nursing	Female	Yes	Yes
6	Mr. R. Nandakumar (Vice-Chairperson, IHEC)	BA., BL	Legal Expert	Male	No	Yes
7	Dr. G. Rajendiran	DM	Clinician (Cardiology)	Male	Yes	Yes

8	Dr. V. Ramamurthy	Ph D	Biotechnology	Male	Yes	No
9	Mrs P Rama	M Pharm	Non-Medical (Pharmacy)	Female	Yes	Yes
10	Dr. P. Sathyan (Chairperson, IHEC)	DO, DNB	Clinician (Ophthalmology)	Male	No	Yes
11	Dr. Seetha Panicker	MD	Clinician (Obstetrics & Gyanaecology)	Female	Yes	Yes
12	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	No
13	Dr. Sudha Ramalingam (Alternate Member-Secretary, IHEC)	MD	Public Health, Epidemiology, Genetics, Ethicist	Female	Yes	No
14	Mrs. Swasthika Soundararaj	MBA	Lay person	Female	No	Yes
15	Dr. D. Vijaya	M Sc, Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

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and only then can they be implemented

f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review

7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

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Yours Sincerely,

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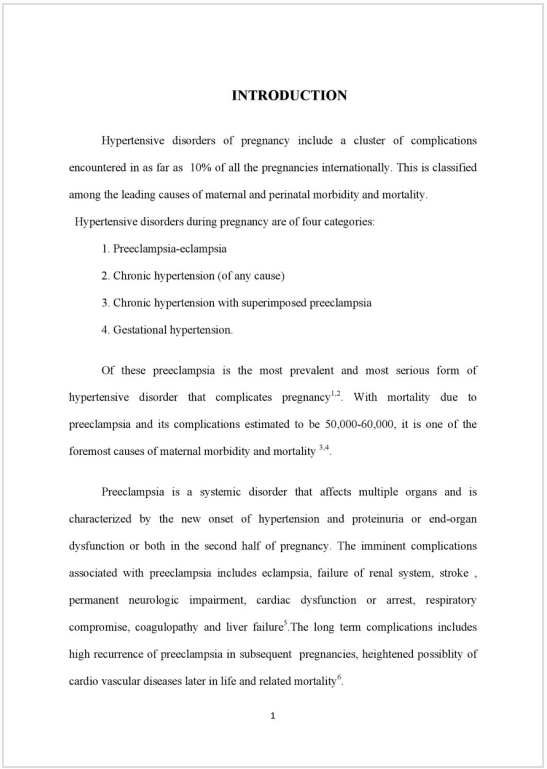

INTRODUCTION

Hypertensive disorders of pregnancy include a cluster of complications encountered in as far as 10% of all the pregnancies internationally. This is classified among the leading causes of maternal and perinatal morbidity and mortality.

Hypertensive disorders during pregnancy are of four categories:

1. Preeclampsia-eclampsia
2. Chronic hypertension (of any cause)
3. Chronic hypertension with superimposed preeclampsia
4. Gestational hypertension.

Of these preeclampsia is the most prevalent and most serious form of hypertensive disorder that complicates pregnancy[1,2]. With mortality due to preeclampsia and its complications estimated to be 50,000-60,000, it is one of the foremost causes of maternal morbidity and mortality [3,4].

Preeclampsia is a systemic disorder that affects multiple organs and is characterized by the new onset of hypertension and proteinuria or end-organ dysfunction or both in the second half of pregnancy. The imminent complications associated with preeclampsia includes eclampsia, failure of renal system, stroke , permanent neurologic impairment, cardiac dysfunction or arrest, respiratory compromise, coagulopathy and liver failure[5].The long term complications includes high recurrence of preeclampsia in subsequent pregnancies, heightened possiblity of cardio vascular diseases later in life and related mortality[6].

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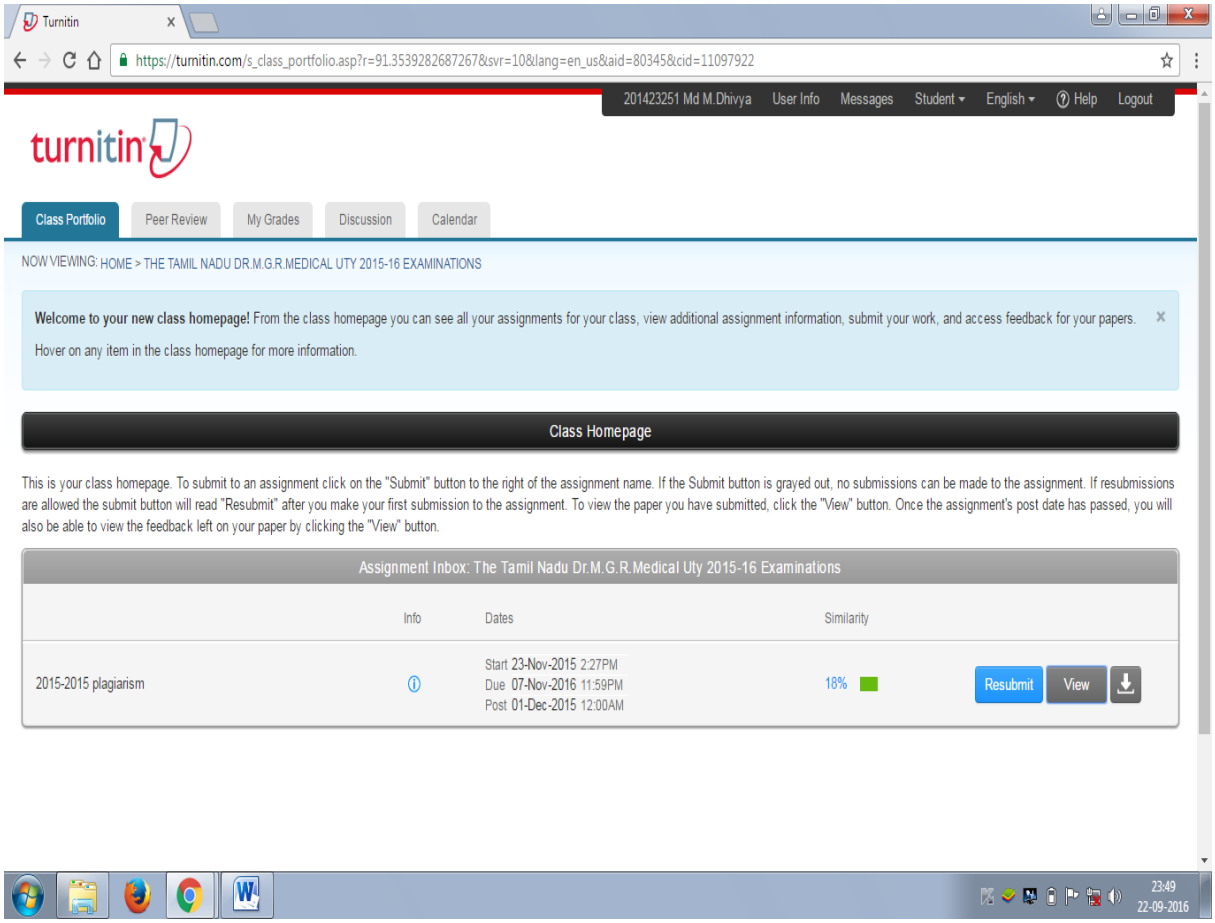
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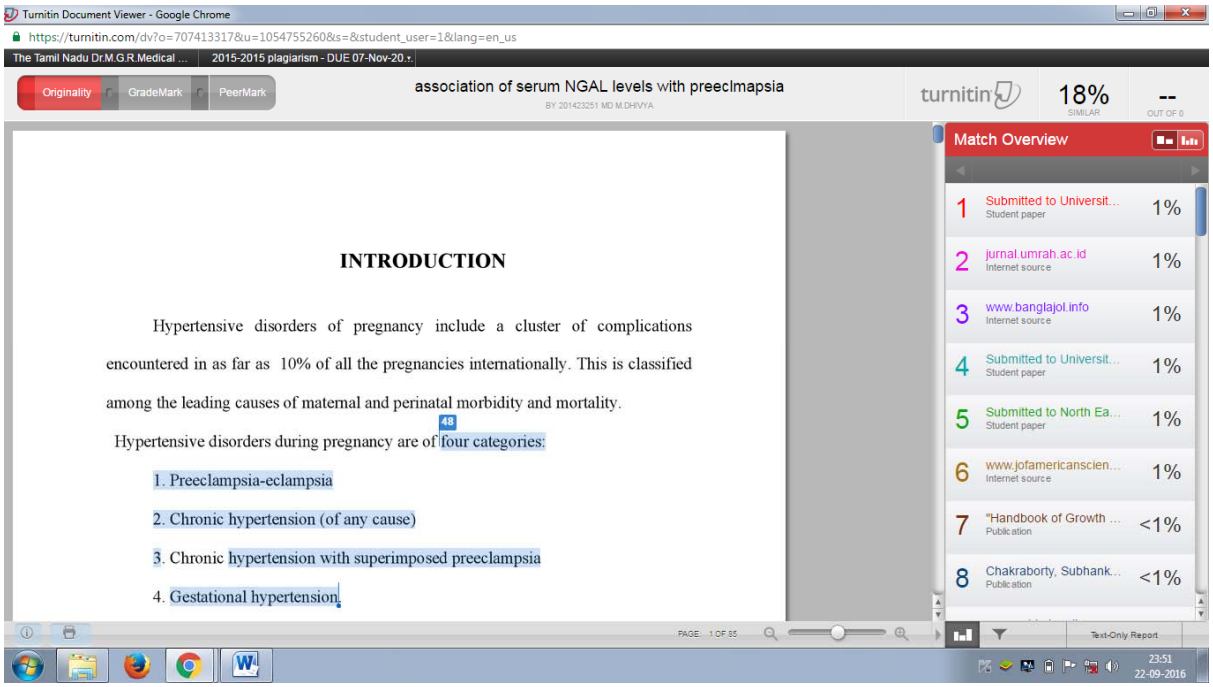
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INTRODUCTION

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The complications in the fetus/newborn includes fetal growth restriction , small for gestational age (SGA) infants, stillbirths , neonatal deaths, neuro developmental impairment such as impaired cognitive skills, motor deficits with fine and/or gross motor delay, cerebral palsy, vision problems, hearing loss, and behavioural and psychological problems[7,8,9,10].

The diagnosis of preeclampsia is based on the increase in blood pressure in a previously normal patient on two occasions after twenty weeks of pregnancy or the presence of proteinuria (>300mg/day).Preeclampsia can be mild or severe. Severe preeclampsia is characterized by more substantial blood pressure elevations (systolic pressure more than or equal to 160 mm Hg, diastolic pressure more than or equal to110 mm Hg),a greater degree of proteinuria (>5g/24 hours) and the presence of symptoms associated with target organ involvement[11].

The pathogenesis of preeclampsia is due to an imbalance between the angiogenic and anti angiogenic factors that leads to the release of inflammatory cytokines; this excessive inflammatory response causes endothelial dysfunction, increased vascular reactivity and coagulopathy that precede the development of symptomatic disease[12]. As kidney is a highly vascular organ, this endothelial dysfunction results in kidney injury.

The development of Acute Kidney Injury (AKI) in pregnancy is termed as Pregnancy-Related AKI (PRAKI). Studies revealed that preeclampsia and eclampsia accounted for most of PRAKI cases[13].There are many biomarker studies for AKI prediction. One of them is serum Neutrophil Gelatinase Associated Lipocalin

(NGAL).NGAL is a 25-kilo Dalton peptide of the lipocalin family. NGAL reduces renal damage by inhibiting cell death and escalating the usual proliferation of kidney tubule cells. So this protein is up regulated in conditions of kidney injury. Rise of NGAL levels has been recognized in the plasma and urine of animal models of ischemic and nephrotoxic acute damage to the renal system. Hence, NGAL is considered to be a contemporary marker for injury to kidney.

But there are only limited number of studies in the pregnant population group to use serum NGAL as a predictor of preeclampsia.

Xiao et al., evaluated a combination of biomarkers for determining AKI with substantial certainity in preeclampsia. Serum cystatin - C, retinol-binding protein in urine, urinary NGAL and kidney injury molecule-1 (urine) levels in the preeclampsia group were higher than the control group. When these markers were united, the sensitivity and specificity for diagnosing AKI were almost 100%[14].

Patel et al., proclaimed that NGAL level was intrinsic to Indian pregnant population with hypertension. Serum NGAL levels in oliguric patients was significantly larger in comparison with non oliguric patients. Serum NGAL level also had a indisputable interrelationship with disease severity including blood pressure level, blood urea nitrogen(BUN), serum creatinine and serum uric acid in hypertensive pregnant patients[15]. This interrelationship is positive.

In the study by Patel et al., the study population included the entire spectrum of hypertensive disorders of pregnancy and it was done in north Indian population. In

this study we have measured the serum NGAL levels in preeclamptic patients in south Indian population and have compared them with control population. If the association between preeclampsia and NGAL levels could be proved in south Indian population also, further studies could be done to use it as a biomarker for preeclampsia thus reducing the morbidity and mortality associated with it.

AIMS AND OBJECTIVES

AIM & OBJECTIVES

Aim:

To compare the levels of serum Neutrophil Gelatinase Associated Lipocalin (NGAL) in preeclamptic women and gestational age matched normotensive controls.

Objectives:

1. To form two groups namely pregnant women with preeclampsia (n=40), normotensive controls that is, pregnant women without preeclampsia (n=40) according to inclusion and exclusion criteria.
2. To collect relevant data and sample for NGAL estimation.
3. To estimate NGAL using ELISA method.
4. To compare the data between 2 groups.
5. To analyze the correlation between the quantitative parameters.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Pregnancy is a physiological state which is an important milestone in a woman's life cycle. Diagnosis of pregnancy can bring about either escalated happiness or profound shock and despair to the mother.

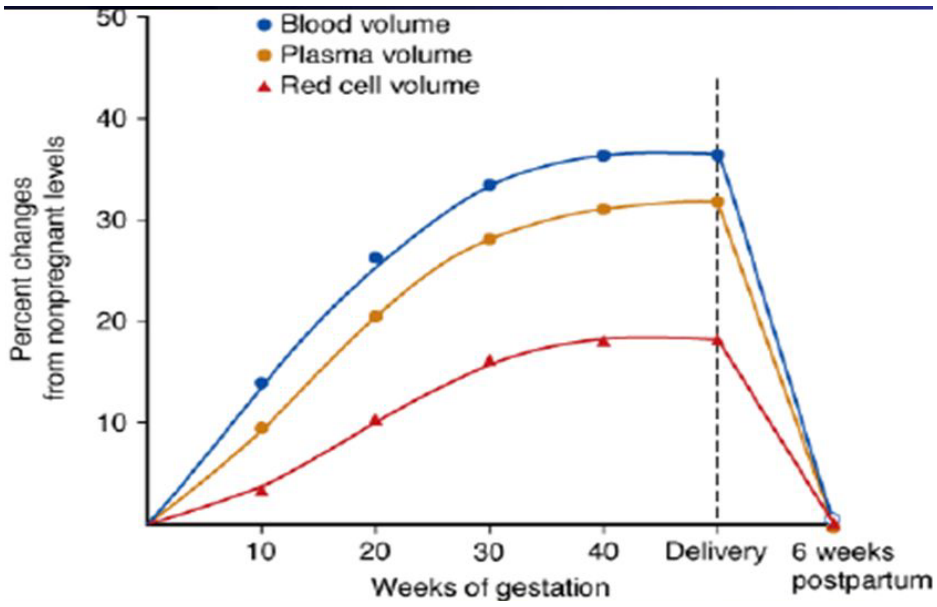
The span of pregnancy which is figured from the day one of last menstrual period is 280 days or 40 weeks[16]. This duration of pregnancy is divided into three trimesters; first trimester-first 12 weeks, second trimester-13-28 weeks, third trimester-29-40 weeks.

The nutrients from the mother and the waste products from the fetus are transferred between them through the placenta which is attached to the uterine wall and establish connection between the maternal and fetal systems through the umblical cord. Apart from this the placenta also has other functions including endocrine functions, barrier functions, immunological functions[16].

When a woman becomes pregnant and as the fetus is developing in the womb, the mother undergoes a myriad of anatomical, physiological and endocrinological changes thus providing a favourable environment for the welfare of the growing fetus. Understanding these physiological changes becomes important in the context of identification of abnormal signs and symptoms. Almost every organ in the maternal system undergoes changes during pregnancy. Among these, changes in cardiovascular system, urinary system, hematological changes are important with respect to preeclampsia.

Hematological changes:

During normal pregnancy, there is an increase in blood volume, plasma volume, erythrocyte count, hemoglobin, leukocyte count and blood coagulation factors[16]. The maternal blood volume begins to increase from 6th week of pregnancy reaching 15% above the pre-pregnancy level by 12th week. Blood volume reaches to 40-45% of the pre-pregnancy level by 32-34 weeks of pregnancy[17].This increase in blood volume provides sufficient nutrients to the placenta and the fetus, protects the mother and fetus from the harmful effects of decreased venous return encountered during the supine and erect postures of the mother. In normal pregnancy there is increase in both coagulation and fibrinolysis. This results in a delicate balance to maintain normal hemostasis[17].



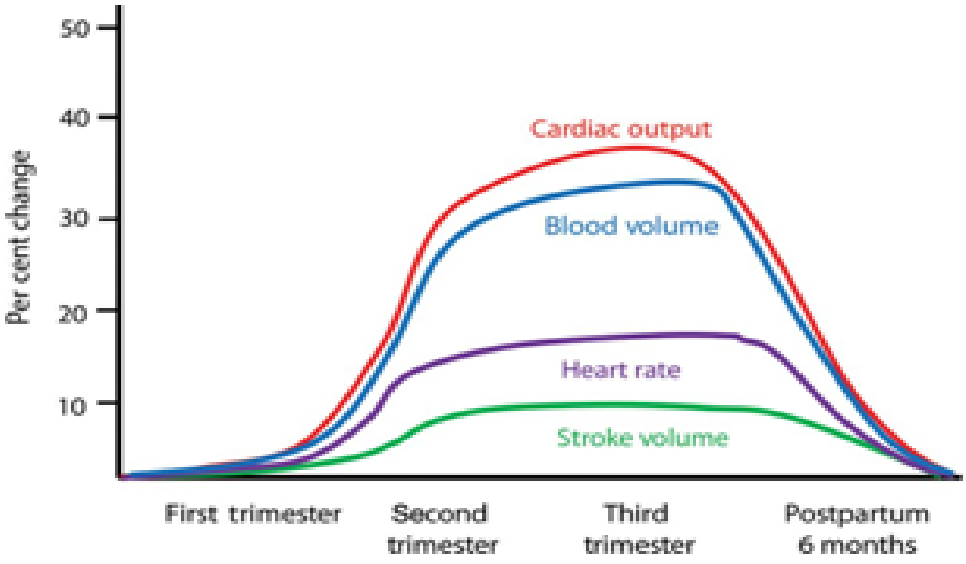
Source-High risk pregnancy-management options, James, Steer, Weiner-4th edition

Cardiovascular System Changes:

There is an increment in cardiac output observed from 5th week of pregnancy which reaches its peak by 30-34 weeks and remains static till term.

Blood pressure:

During pregnancy there is a decrease in blood pressure which reaches its nadir by 24-26 weeks[17]. This is due to decrease in systemic vascular resistance which in turn is effected by prostaglandins, progesterone, nitric oxide, atrial natriuretic peptide. Even though there is a marked increase in cardiac output, the blood pressure is decreased due to fall in systemic vascular resistance. Ultimately there is an overall fall in diastolic blood pressure, mean arterial pressure by 5-10 mm of Hg[16].

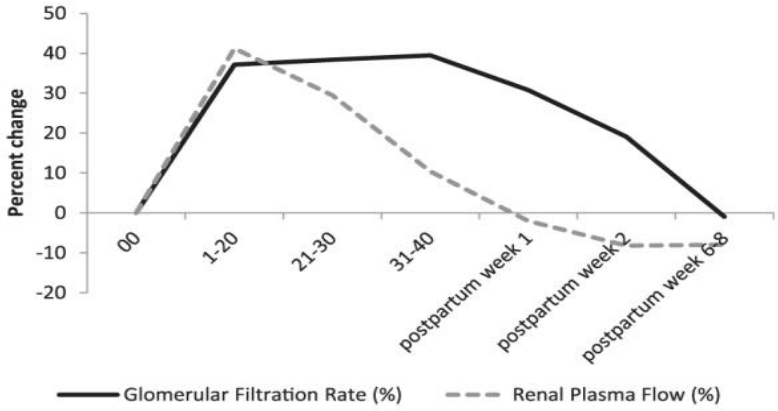


Source-William's obstetrics-Kenneth .J.Leveno-24th edition.

Due to the pressure exerted by the gravid uterus on the common iliac vein there is an increase in femoral venous pressure. This along with the distensiblity of the veins contribute to the development of physiological edema of pregnancy which subsides on taking rest[16].

Changes in the urinary system:

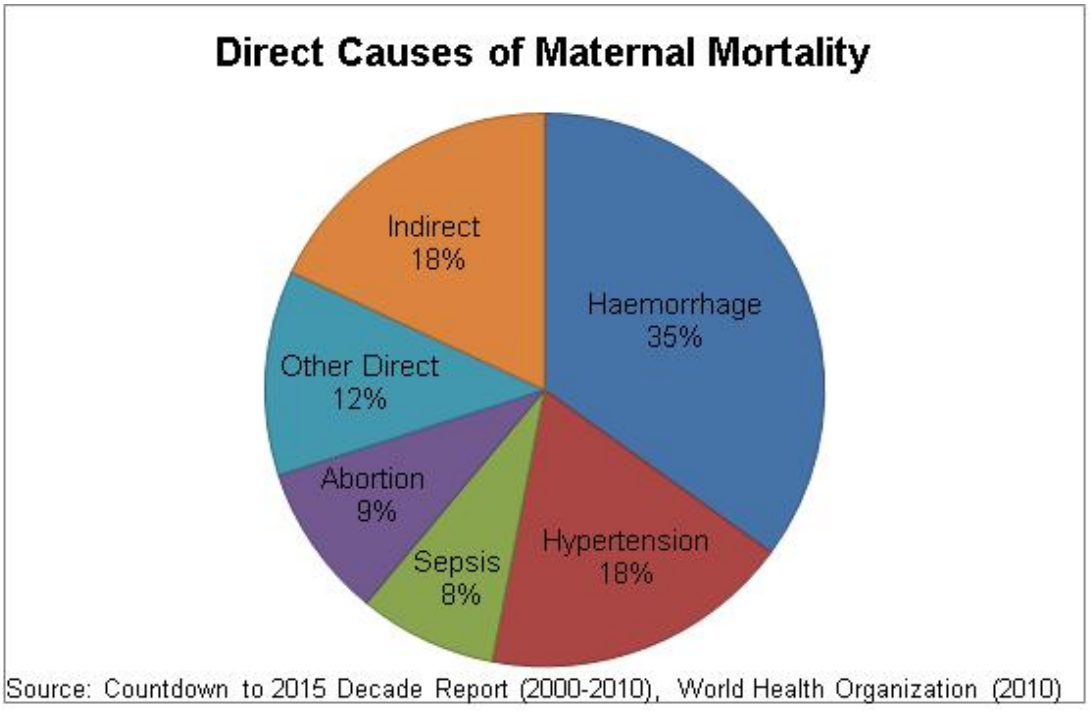
The renal changes during pregnancy can be divided as anatomical and physiological. The anatomical changes include hydronephrosis which is seen in about 43-100% of normal pregnant women. It is observed that the length of the kidney also increases by 1-1.5 cm. There is a 30% increase in the overall volume of the kidney. There is an increase in renal plasma flow by 50-75%. The peak in the renal plasma flow is attained by 16 weeks and is static till 34 weeks after which it falls by 25%. There is an increase in glomerular filtration by 50% throughout pregnancy. Both these factors results in decreased maternal levels of creatinine, blood urea nitrogen and uric acid[16].



Source: Katharine, Richard, Renal Physiology of Pregnancy, Adv Chronic Kidney Dis. 2013 May; 20(3): 209–214.

High risk pregnancy:

Pregnancy is considered an exclusive physiological experience in a woman's life time. But when there is a precedent illness or an unforeseen disease either in the mother or the developing embryo, it will complicate the pregnancy. Risk can be designated as the chances of an unfavourable result or a factor that will increase the probability of an adverse outcome. A pregnancy is said to be of high risk when the chances of an unfavourable result for the mother or baby is increased when compared with general pregnant population[18]. One of the causes for high risk pregnancy is hypertensive disorders.



Hypertensive disorders of pregnancy:

Hypertensive disorders are present in 5-10% of all pregnancies. According to WHO,16% of all maternal deaths are due to hypertensive disorders[17]. Working group

of National High Blood Pressure Education Programme (NHBPEP) has divided hypertensive disorders of pregnancy into four groups[17]. They are-

i) Gestational hypertension

ii) Preeclampsia and eclampsia syndrome

iii) Chronic hypertension of any etiology

iv) Preeclampsia superimposed on chronic hypertension.

It is determined that a woman has hypertensive disorders peculiar to pregnancy when systolic pressure greater than or equal to 140 mm of Hg along with or without diastolic blood pressure greater than or equal to 90 mm of Hg[18].

Preeclampsia and eclampsia:

The incidence of preeclampsia varies from 5-15%[16].Worldwide the maternal mortality due to preeclampsia is around 50,000-60,000 annually, making it one among the common causes of mortality in the mother[19]. Preeclampsia can be considered as a syndrome specific to pregnancy characterized by variable degrees of placental dysfunction and maternal response in the form of systemic inflammation. It is a multisystem disorder of undetermined etiology characterized by the evolution of hypertension with protein in urine after twenty weeks of gestation in a previously non proteinuric and normotensive woman. The pathogenesis and pathophysiology of preeclampsia involves many genetic and environmental factors and is poorly understood[19].

There are two types of preeclampsia-early onset and late onset, based on the point of time of onset of clinical features. In the first type, the clinical signs appear

before 33 weeks of pregnancy and in the second type, the features appear at or later than 34 weeks of pregnancy. Although the late onset type forms major type, the maternal and neonatal mortality and morbidity are higher in the early onset type[19].

Diagnostic criteria:

According to the guidelines recommended by American College of Obstetricians and Gynaecologists, task force 2013 and also by Society for Obstetric Medicine Australia and New Zealand (SOMANZ)[17,18],a clinical diagnosis of preeclampsia can be made when

- Blood Pressure more than or equal to 140/90 mm of Hg in a previously normotensive woman and any one or more of the following:
- Proteinuria ≥ 300 mg/24 hours or urine protein creatinine ratio ≥ 0.3
- Serum creatinine > 1.1 mg/dL or if the value is doubled in a patient with no prior renal disease
- Thrombocytopenia –platelet count<1,00,000/µl
- Abnormal Aspartate transaminase and /or Alanine transaminase >50 IU/L(twice the normal)
- Raised bilirubin > 25 IU/ml and /or severe epigastric or right upper quadrant pain
- Convulsions ,persistent atypical severe headache
- Persistent visual disturbances (photopsia, scotomata, cortical blindness, retinal vasospasm)
- Stroke, hyperreflexia with sustained clonus
- Pulmonary edema

- Placental abruption
- IUGR and/or signs of fetal distress

Although not recommended by ACOG guidelines, serum uric acid level measurement for the diagnosis of preeclampsia is widely practiced in many set ups. Even though elevated uric acid levels are seen in only 80% of the pregnancies with preeclampsia, serum uric acid levels of more than or equal to 6 mg/dl is considered asa supportive evidence for the diagnosis of preeclampsia.

Risk factors for preeclampsia[18]:

- Primi gravida
- When maternal age is on either end of the extreme
- Multifetal gestation
- Preeclampsia in a preceding pregnancy
- Obesity and insulin resistance
- Chronic hypertension and /or renal disease
- Maternal chronic infections
- Family history of hypertension /preeclampsia

Etiopathogenesis :

Etiology :

There are two stages in the development of preeclampsia. In the first asymptomatic stage there is abnormal placentation which may be because of the ischemia[20]. In the second stage, the soluble factors from the placenta enters the maternal circulation which will culminate in endothelial dysfunction and the clinical

symptoms[20].There are various mechanisms that have been proposed to explain development of preeclampsia by various causes. They include[3];

a. Abnormal trophoblastic invasion of uterine vessels.

b. Immunological maladaptations between maternal, placental, fetal tissues.

c. Maternal maladaptations to cardiovascular or inflammatory changes of normal pregnancy

d. Genetic factors like inheritance of predisposing genes and epigenetic influences[18]

A. Abnormal trophoblastic invasion[16,17,18]:

The processes of implantation and placentation are vital in the continuation of normal pregnancy. After implantation, that is during the process of placentation, there is physiological hypoxia which in turn stimulates the proliferation of trophoblasts[21].

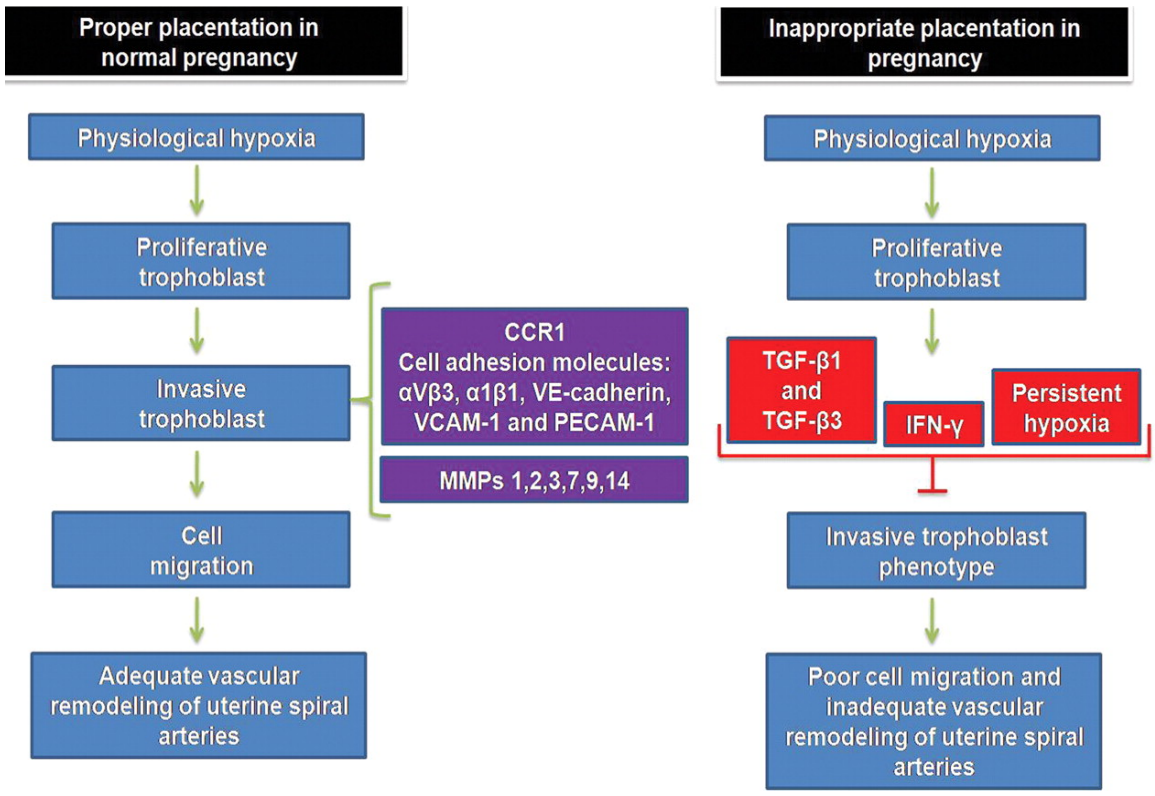
During the process of implantation of the embryo, the trophobalstic cells surrounding the embryo differentiates into extravillous cytotrophoblasts and syncytiotrophoblasts[19]. The trophoblasts can acquire the property of invasiveness after the expression of chemokine receptors like CCR-9 and the cell adhesion molecules like vascular cell adhesion molecule-1,platelet endothelial cell adhesion molecule-1,vascular endothelial cadherin[21].

Apart from the expression of these cell surface molecules the invasive trophoblasts should have the ability to invade the extracellular matrix. They accomplish this by elaborating matrix metalloproteinases-MMP-1,2,3,9,14[21]. In addition, the paracrine factors like activin-A from maternal circulation and

transforming growth factor-beta(TGF-β) are also important in the process of trophoblastic invasion[21].

Activin-A favours trophoblastic invasion whereas transforming growth factor-beta inhibits trophoblastic invasion. There are three types of transforming growth factors(TGF)-β1,β2,β3.TGF-β1 and β2 are expressed by the decidual cells whereas TGF- β3 is expressed only by the cells of the immune system[21]. This TGF- β3 is found in high concentrations in patients with preeclampsia. These TGFs inhibit trophoblastic invasion by binding with the matrix metalloproteinases[21].

Abnormal trophoblastic invasion of preeclampsia:



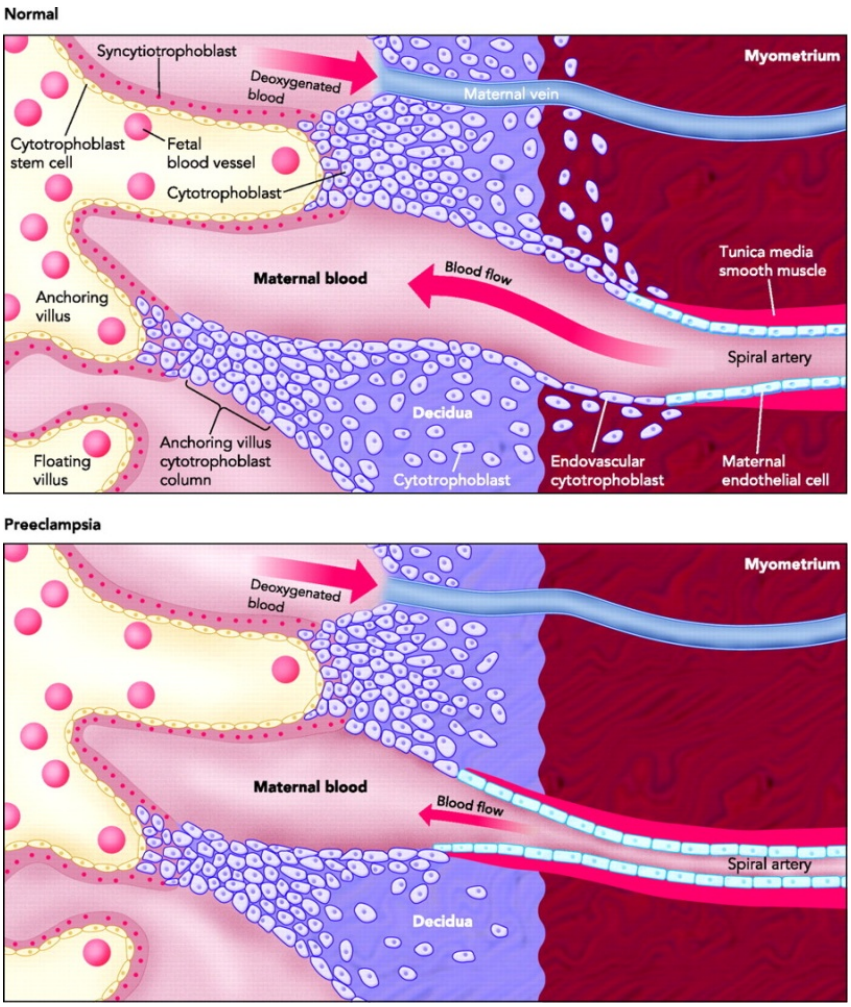
Source: An immunological insight into the origins of pre-eclampsia, Oxford Journals Medicine & Health, Human Reproduction Update, Volume 16, Issue 5,Pp. 510-524

The cytotrophoblasts form the extravillous trophoblasts which invade the myometrium and the spiral arteries[19]. Normally by 12th week of pregnancy there is a first wave of cytotrophoblastic invasion of the maternal spiral arteries in the decidual region. Then by 16-18 weeks, there is a second wave of cytotrophoblastic invasion in the spiral arteries up to the inner one third of myometrium.

The invasion of the spiral arteries by these extra villous trophoblasts causes remodelling of the spiral arteries with loss of elastic lamina, smooth muscle cells and endothelial cells and as a result the vessels are lined by epithelial cells[17]. This results in the conversion of low flow, high resistance vessels into high flow, low resistance ones which are essential for normal fetal growth.

This migration of the extravillous trophoblasts into the spiral arteries is affected by factors like growth factors, cytokines, oxygen tension and the immune cells like macrophages and decidual natural killer cells[17].

In preeclampsia, this second wave of cytotrophoblastic invasion does not occur, thus decreasing the blood flow to the feto-placental unit. This defect in the spiral arteries leads to additional slump in the previously existing hypoxic condition of the pregnancy, thus intensifying it.

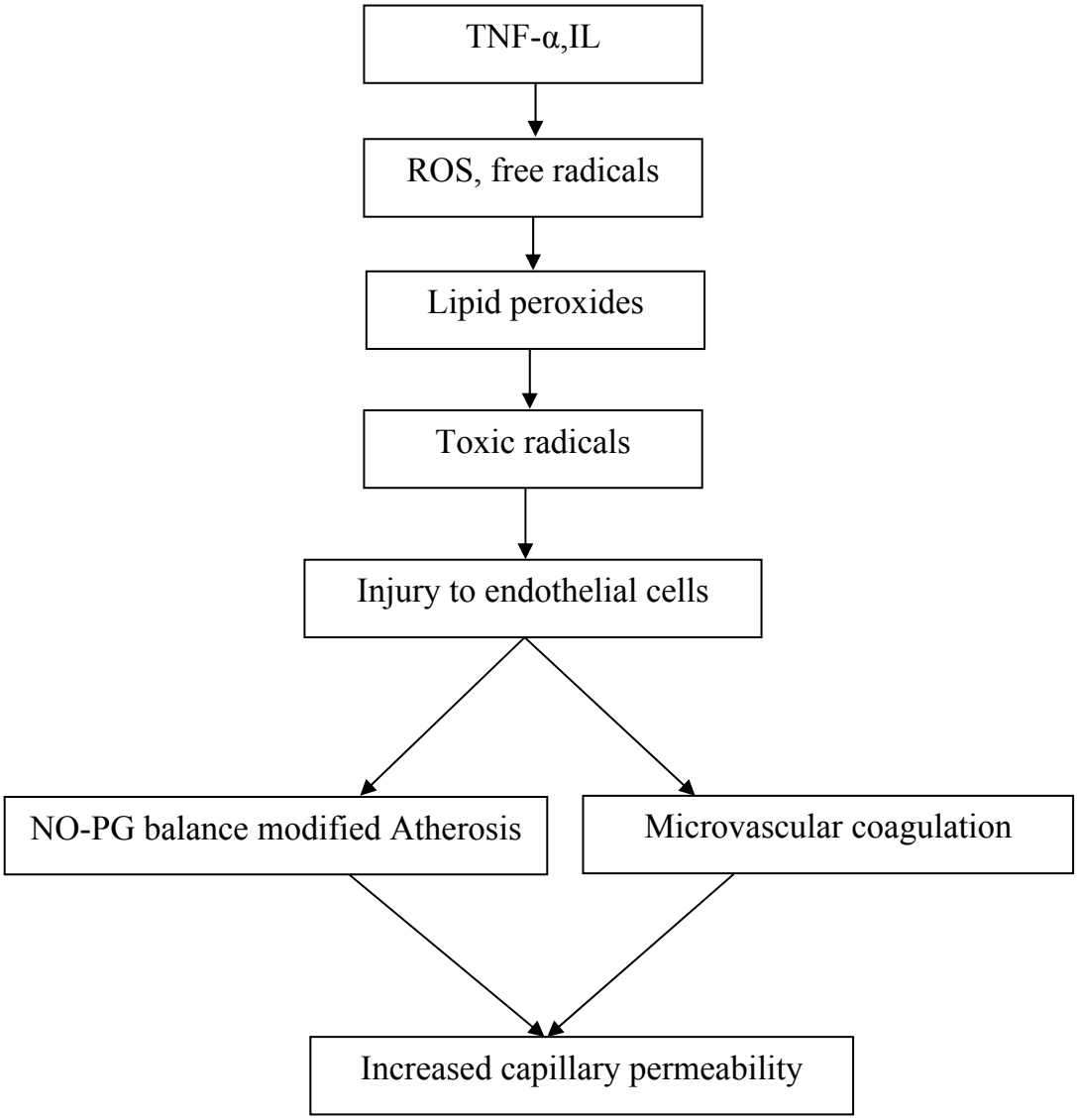


Source: Preeclampsia: The Role of Angiogenic Factors in Its Pathogenesis, Aliceng, Sarosh, S. Ananth, Physiology Published 9 June 2009 Vol. 24 no. 3, 147-158

Endothelial cell activation[17,18]:

Because of abnormal trophoblastic invasion, there are ischemic changes in the placenta which leads to release of placental factors, which in turn results in the release of anti-angiogenic, metabolic and other inflammatory mediators that provoke endothelial cell injury.

Endothelial cell dysfuncion is due to prostacyclin(PGI_2), thromboxane- A2 imbalance, cytokines like TNF-α & Interleukins.



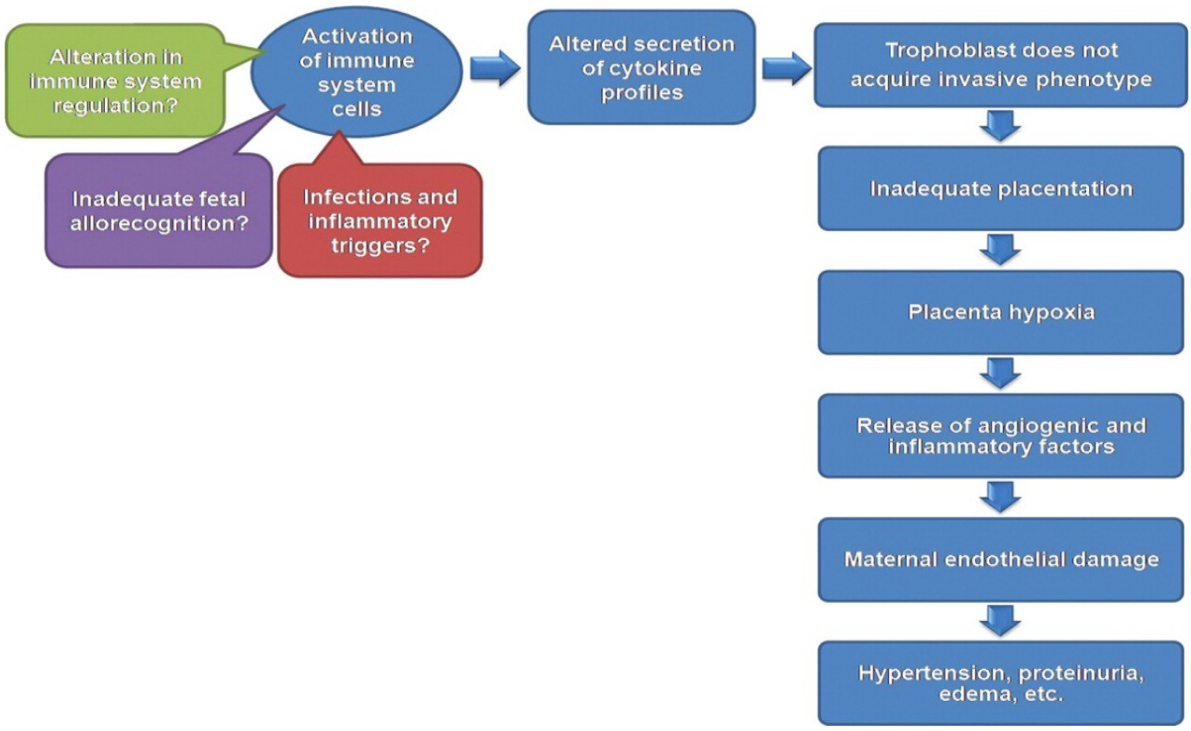
Immunological factors in the development of preeclampsia:

The immunological processes in the development of preeclampsia can be divided into two stages:

i. Due to under expression of Human Leukocyte Antigen (HLA-G) by the trophoblasts, there is inadequate stimulation of the decidual natural killer cells.

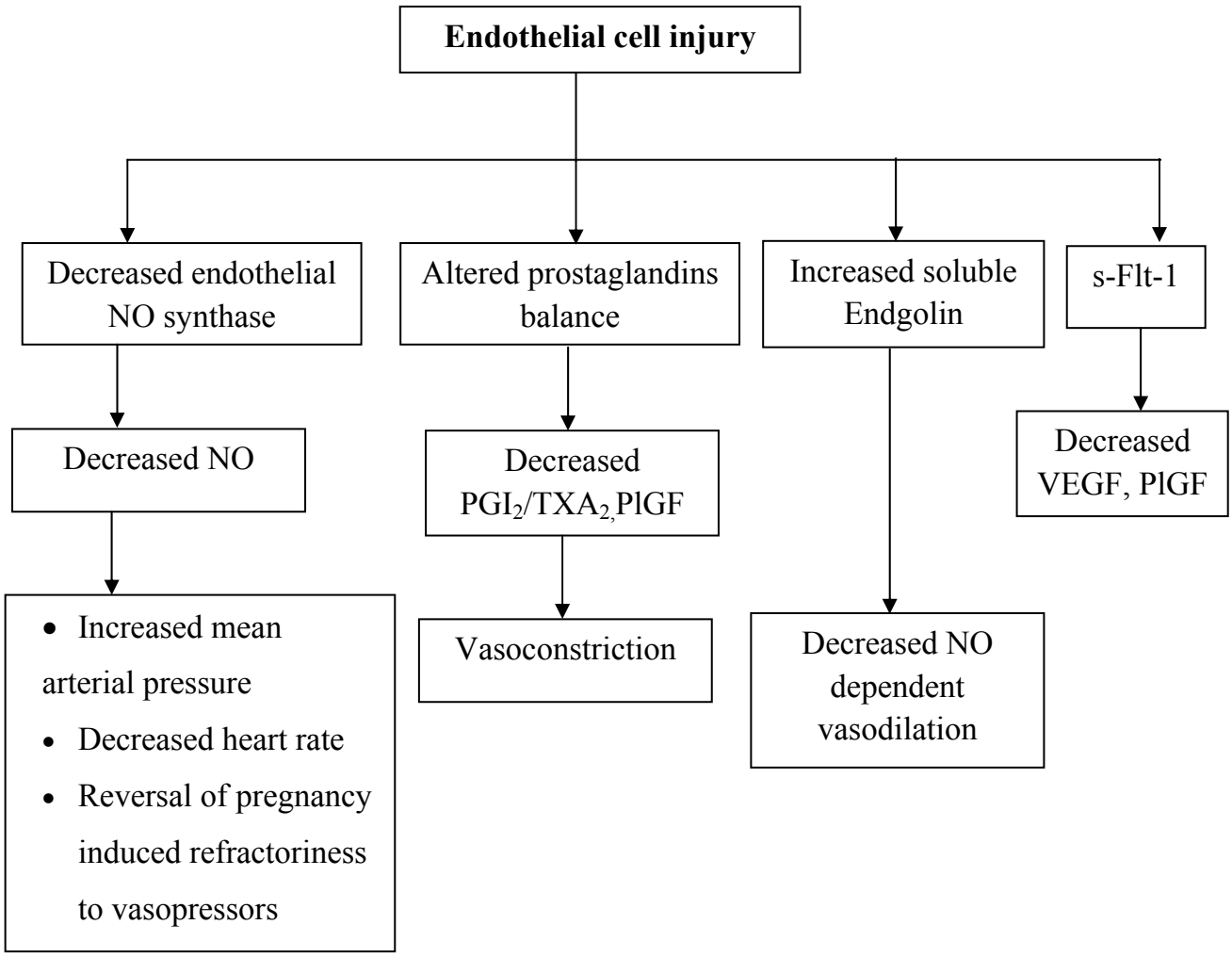
These decidual natural killer cells are responsible for the production of immune-regulatory cytokines and angiogenic factors. All these factors leads to poor trophoblastic invasion of the spiral arteries with resultant hypoperfusion[21].

ii. The release of necrotic and/or apoptotic syncytiotrophoblasts into the maternal circulation acts as an inflammatory stimulus and results in an systemic inflammatory response involving the leukocytes and endothelium[21].



Source: An immunological insight into the origins of pre-eclampsia, Oxford Journals Medicine & Health, Human Reproduction Update, Volume 16, Issue 5,Pp. 510-524

Pathogenesis[17]:



s-Flt-1—soluble Fms like tyrosine kinase-1, PlGF-placental growth factor, VEGF-vascular endothelial growth factor

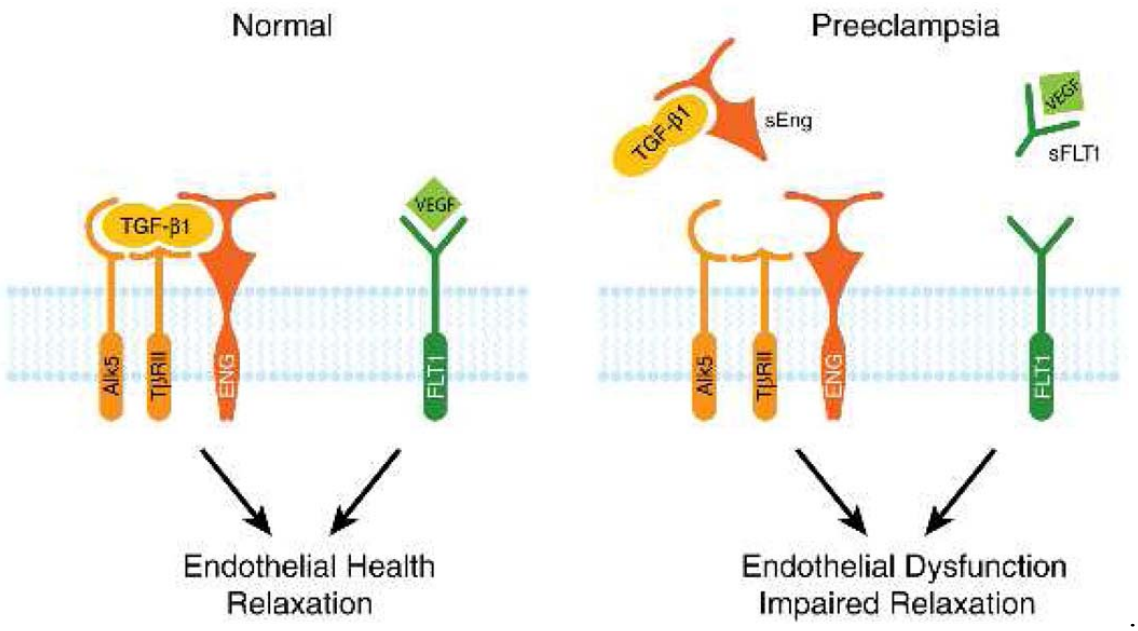
Angiogenic and anti-angiogenic factors in preeclampsia:

The trophoblastic cells secrete growth factor of placenta and growth factor of vascular endothelium which are necessary for angiogenesis in the placenta. Apart from these, transforming growth factor beta is also an angiogenic factor[22].The anti-angiogenic factors include vascular endothelial growth factor receptor-1& 2 and

endoglin. A soluble form of endoglin is formed by the action of matrix metalloproteinase with the cleavage of extracellular domain[22]. This soluble endoglin acts as an anti-angiogenic factor by binding to the angiogenic factor transforming growth factor beta (TGF-β).

Vascular endothelial growth factor receptor-1 is also known as Fms- like tyrosine kinase-1(Flt-1) which is membrane bound. A truncated form of Flt-1 is called s-Flt-1, is formed by the alternative splicing of the Flt-1[22]. This s-Flt-1 contains the extracellular domain but does not contain the transmembrane and intracellular domain. It exerts its anti-angiogenic action by binding to vascular endothelial growth factor and placental growth factor[22].

Vascular endothelial growth factor and placental growth factor are responsible for the maintenance of normal endothelial cells. Vascular endothelial growth factor-2 is a kinase insert domain receptor. The balance between the angiogenic and anti-angiogenic factors is essential for the normal placental vascularisation which is disrupted in preeclampsia[22].



Source: Angiogenic Factors and Preeclampsia Sharonand S. Ananth, Semin Nephrol. 2011 Jan; 31(1): 33–46.

The patho physiological and etiological pathways appear to differ for early and late onset preeclampsia. The early type preeclampsia is characterized by growth restriction of the fetus in utero which may be due to the incomplete transformation of spiral arteries[17]. This leads to hypoperfusion of the placenta and intrauterine growth restriction. But in late onset preeclampsia, the diameter of the spiral arteries is altered only a little and there is no evidence of placental hypoperfusion or intrauterine growth restriction[17].

Pathophysiology:

Cardiovascular system[17]:

Due to endothelial cell activation, there is a generalized vasoconstriction and leakage of plasma into interstitial space resulting in hemo concentration which in turn results in decreased cardiac preload.

Due to hypertension there is increased cardiac after load. This decreased cardiac preload and increased after load results in decreased cardiac output.

Hematological changes:

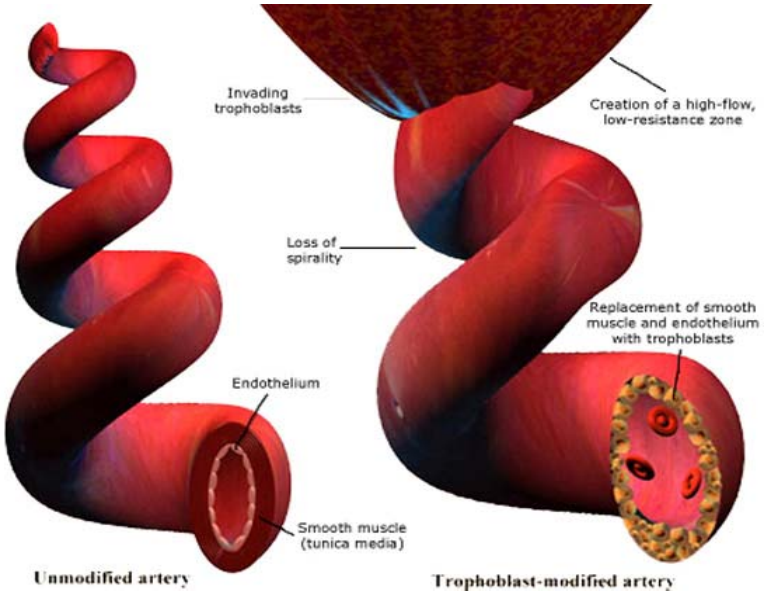
In preeclampsia, there is thrombocytopenia with the platelet count less than 1,00,000/μl and this indicates the presence of severe disease.

Hemolysis:

The hemolysis that is characteristic of preeclampsia is microangiopathic hemolysis. This typical hemolysis is due to endothelial disruption with platelet adherence and fibrin deposition seen in preeclampsia.

Coagulation :

There is increased factor VIII consumption, increased fibrino peptides A and B, decreased anti thrombin-III and protein C and S which is responsible for the hypercoagulable state in preeclampsia.



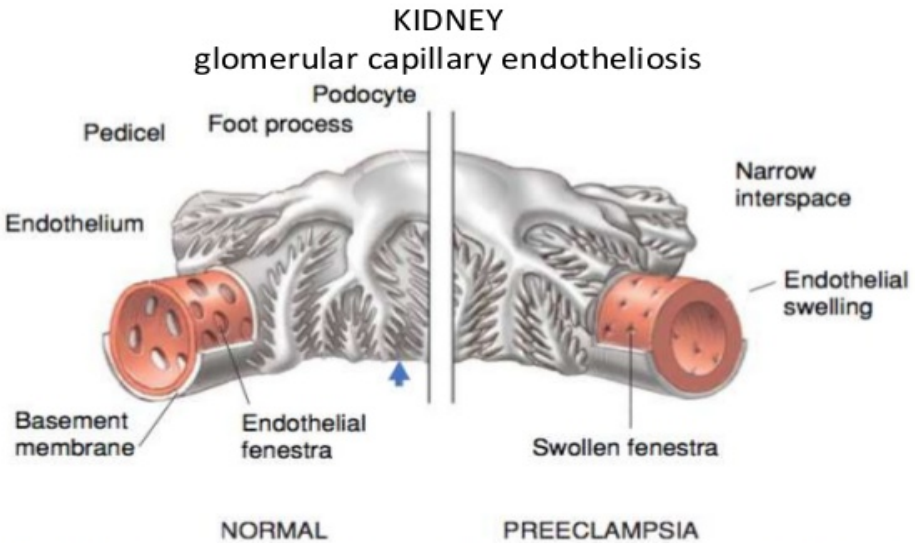
Source:http://www.rcdrg.sgul.ac.uk/research/trophoblasts

Kidney[17]:

Endothelial cells in the glomerular capillaries are swollen. This endothelial swelling and vacuolisation accompanied by the obstruction of the capillary space is referred to as glomerular capillary endotheliosis. These changes are also accompanied by sub endothelial fibrin deposition and loss of endothelial fenestrae.

This is due to the unavailability of angiogenic factors which characterizes severe disease. This typical morphology seen in preeclampsia is referred to as thrombotic microangiopathy (TMA) which is characteristic of preeclampsia but not pathognomic of it[23]. The presence of thrombotic microangiopathy is due to the endothelial injury of the small blood vessels in the form of glomerular endotheliosis[23].

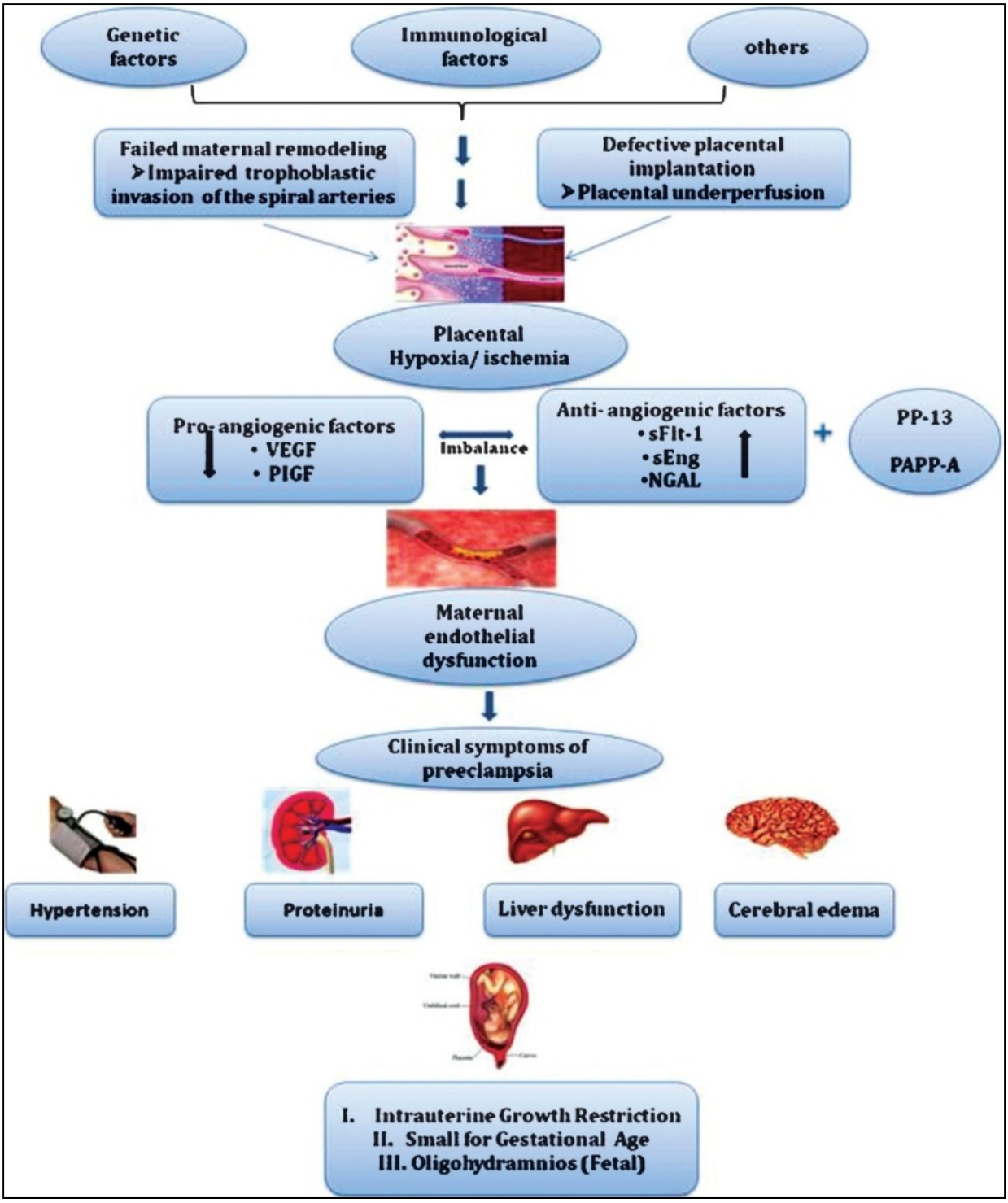
Preeclampsia is characterized by decreased renal blood flow and glomerular filtration rate. Decreased GFR leads to elevated serum creatinine, uric acid levels. Loss of function of podocytes results in proteinuria which is a hallmark in preeclampsia.



Source-High risk pregnancy-management options-James,Steer,Weiner-4th edition

Liver[18]:

Symptomatic involvement of liver in preeclampsia indicates severe disease. In severe preeclampsia, there are large deposits of fibrin like material in the hepatic sinusoids which results in hepatic capsular distension and upper epigastric pain. This will result in hepatic infarction, periportal hemorrhagic necrosis and elevated levels of the liver enzymes, alanine transaminase and aspartate transaminase(ALT and AST).



Source: Biomarkers for the management of pre-eclampsia in pregnant women Lakshmi Tanuja et al, Indian Journal of Medical Research, 2013,Volume : 138,Issue : 1,Page : 60-67

HELLP syndrome[16]:

HELLP is an cipher for Hemolysis, Elevated Liver enzymes and Low platelet count. It is seen in 10-15% of preeclampsia patients. It is characterized by nausea, vomiting, epigastric or right upper quadrant pain, alanine transaminase, aspartate transaminase level more than 70 IU/L, LDH more than 600 IU/L, bilirubin more than 1.2 mg/dl.

The hemolysis that is seen in HELLP syndrome is due to micro angiopathic haemolytic anemia[24]. In the peripheral smear, there is presence of fragmented RBCs or schizocytes and burr cells which are contracted RBCs with spicula[24].

The hemoglobin released by RBC lysis is bound by haptoglobin and metabolised by the liver to bilirubin with the resultant decrease in haptoglobin level and increase in bilirubin level[24].

The damaged and activated endothelium which releases the Von Wille brand Factor multimers leads to consumption thrombocytopenia[24].The platelets are activated and they adhere to the damaged endothelial cells which results in their rapid turn over and decreased platelet count(less than 1,00,000/μL)[24].

The lesion in the liver that characterizes HELLP syndrome is periportal and focal parenchymal necrosis with hyaline like fibrin deposits in the hepatic sinusoids[24].

The presence of one or two components of the HELLP syndrome is called partial HELLP syndrome[24].

Brain:

Auto regulation of cerebral blood flow which is a protective mechanism against alterations in cerebral perfusion pressure is lost in preeclampsia and this results in cerebral edema[17]. Other changes seen in brain in preeclampsia includes capillary thrombosis, infarction, intra ventricular and parenchymal haemorrhages and necrosis.

The clinical features include headache, blindness, scotomata, blurred vision, diplopia and convulsions.

Clinical types of preeclampsia[16]:

Preeclampsia can be mild or severe and this classification is arbitrary.

Mild preeclampsia :

Preeclampsia is said to be mild, when the blood pressure is more than or equal to 140/90 mm of Hg but less than 160/110 mm of Hg without significant proteinuria or signs of any other end organ damage.

Severe preeclampsia:

Preeclampsia is said to be severe when one or more of the following symptoms are present.

Persistent systolic Blood pressure ≥ 160 mm of Hg or diastolic Blood pressure ≥ 110 mm of Hg

1. Protein excretion > 5g/24 hours
2. Oliguria (urine output <400 ml/24 hours)
3. Platelet count < 1,00,000/µl

4. HELLP syndrome
5. Cerebral/visual disturbances
6. Persistent severe epigastric pain
7. Retinal haemorrhages, exudates, papilledema
8. Pulmonary edema
9. Intra uterine growth retardation (IUGR) of fetus.

Effects of preeclampsia on the fetus[18]:

The effects of preeclampsia on the fetus depends on the gestational age at the time of onset of disease. Outcomes during delivery are favourable in patients having with mild preeclampsia manifesting after 36 weeks of pregnancy.

Perinatal morbidities and deaths are elevated in patients who manifest preeclampsia prior to 33 weeks of gestation.

The important effect of preeclampsia is intra uterine growth retardation and preterm (<32 weeks of gestation) delivery[18]. Other complications include intra uterine death and asphyxia[16].

Treatment[16] :

1. Rest –advising the patient to take bed rest in left lateral position.
2. Anti hypertensives – drugs like methyldopa, labetalol, nifedipine, hydralazine

Preeclampsia complicated with grand mal seizures is eclampsia and the drug of choice for the treatment of eclampsia is magnesium sulphate.

Other hypertensive disorders of pregnancy[16,17]:

Gestational hypertension:

A patient is said to have gestational hypertension when the BP is more than or equal to 140/90 mm of Hg for the first time in pregnancy after 20 weeks of gestation without proteinuria.

Chronic hypertension:

A patient is said to have chronic hypertension if she is a known hypertensive before pregnancy or hypertension diagnosed first time before 20 weeks of gestation.

Preeclampsia superimposed on chronic hypertension:

The occurrence of new onset proteinuria in a woman with pre-existing chronic hypertension is called preeclampsia superimposed on chronic hypertension.

Acute renal damage in preeclampsia:

Acute renal damage is defined as an increase in serum creatinine of 1.5 times the normal baseline within 2-7 days or if there is oliguria i.e if the urine output is 0.5 ml/kg/hr for 6 hours. If the acute kidney injury is diagnosed in pregnant women, it is called pregnancy related acute kidney injury (PRAKI).It is seen in 1 in 20,000 pregnancies[25]. The PRAKI is one of the most common causes of acute kidney injury(AKI).

Pregnancy related acute kidney injury is defined as acute increase in serum creatinine of 0.1-0.5 mg/dl from the baseline value or serum creatinine > 1-2 mg/dl. Preeclampsia and eclampsia are the most common causes of PRAKI.

The causes for AKI in preeclampsia includes[26]:

Primary changes:

1. Glomerular endotheliosis
2. Decrease in GFR
3. Decrease in renal plasma flow.

Secondary effects:

1. Intravascular volume depletion
2. Vasoconstriction
3. Activation of inflammatory cascade
4. Activation of coagulation cascade.

Biomarkers of acute kidney injury :

The various biomarkers of acute kidney injury includes[27]:

S.No	Biomarker type	Biomarker
1	Functional markers	i.Serum creatinine ii.Cystatin -c
2	Up regulated proteins	i.NGAL ii.KIM-1 iii.L-FABP iv.IL-18
3	Proteins with low molecular weight	Urine cystatin-c
4	Enzymes	NAG α-GST, π-GST GGT,AP

i. NGAL-Neutrophil Gelainase Associated Lipocalin

ii. KIM-1-Kidney Injury Molecule-1

iii. L-FABP-Liver Fatty Acid Binding Protein

iv. NAG-N-Acetyl Glucosaminidase

v. α-GST, π-GST-Glutathione –S-Transferase

vi. GGT-Gamma Glutamyl Traspeptidase

vii. AP-Alkaline Phosphatase

Serum creatinine:

Serum creatinine levels are correlated with the muscle mass. To find out the extent of kidney injury using creatinine levels, GFR is calculated using equations and these equations are imprecise when GFR > 60ml/minute. This has poor predictive accuracy in the early stages of acute renal damage.

Serum cystatin-c:

It is a single chain basic protein which is non glycosylated and has a molecular weight of 13,360 kDa. It is synthesized by all cells with nucleus. This is a cysteine protease inhibitor which is excreted by filtration by glomerulus and is neither secreted nor reabsorbed by the tubules. It may detect acute renal failure 1-2 days earlier than that of creatinine. But cystatin-c levels are altered by gender, body mass index, abnormal thyroid function and malignancies.

Up regulated proteins:

Kidney Injury Molecule-1(KIM-1):

KIM-1 is a membrane glycoprotein and this is involved with the phagocytosis of the damaged epithelial cells in the tubular lumen. Its level is increased in urine after a renal injury.

Liver Fatty Acid Binding Protein (L-FABP):

When there is a hypoxic tissue injury, there will be release of lipid peroxidation products and intracellular unsaturated fatty acids which are bound by L-FABP. Urinary L-FABP is a biomarker for Acute kidney injury(AKI).

IL-18:

IL-18 is an essential mediator in the process of acute kidney injury. So its levels are increased in acute kidney injury in urine.

Enzymes:

N-Acetyl Glucosaminidase:

This enzyme is produced by the lysosomes of renal tubular epithelial cells. The level of this enzyme is increased in acute kidney injury.

α-GST, π-GST(Glutathione –S-Transferase):

These are the detoxification enzymes and are not normally present in urine. After acute kidney injury, α-GST is found in the proximal part of nephron and π-GST is found in the distal parts of the nephron. But these enzymes are not good as markers of acute renal injury.

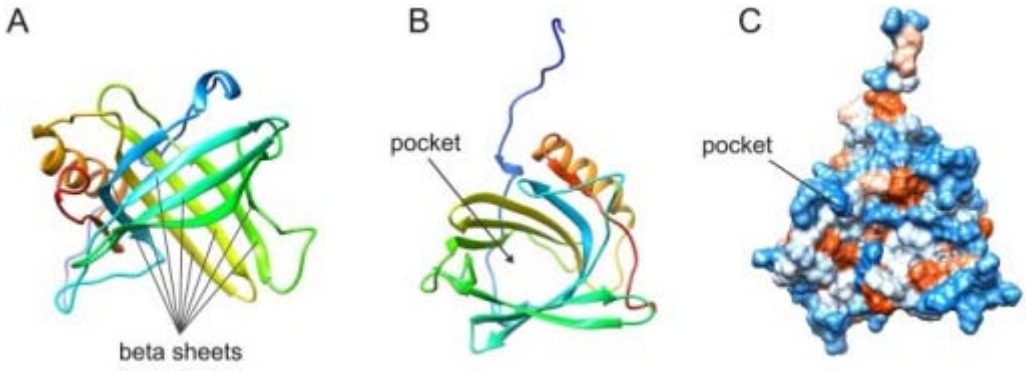
GGT & AP(Gamma Glutamyl Transpeptidase & Alkaline Phosphatase):

These enzymes are present in the brush border of epithelial cells of renal tubules and are excreted into the urine when there is acute kidney injury.

Neutrophil Gelatinase Associated Lipocalin (NGAL):

Neutrophil Gelatinase Associated Lipocalin (NGAL) is also known as lipocalin-2,siderocalin,LCN-2,24p3,migration stimulating factor inhibitor (MSFI), Human neutrophil lipocalin (HNL),α-1 microglobulin related protein,uterocalin[28].

NGAL is synthesized in the bone marrow during myelopoiesis and stored in the neutrophils. NGAL belongs to lipocalin family of proteins. These are small secreted glycoproteins which binds to small hydrophobic molecules. The proteins in the lipocalin family do not have much sequence similarity except for short stretches of aminoacids which are three in number[28]. Based on these structurally conserved regions, there are two families of lipocalins, kernel lipocalins and outlier lipocalins. Kernel lipocalins have all the three motifs and outlier lipocalins have only two of the motifs. NGAL belongs to kernel lipocalins subtype[28].



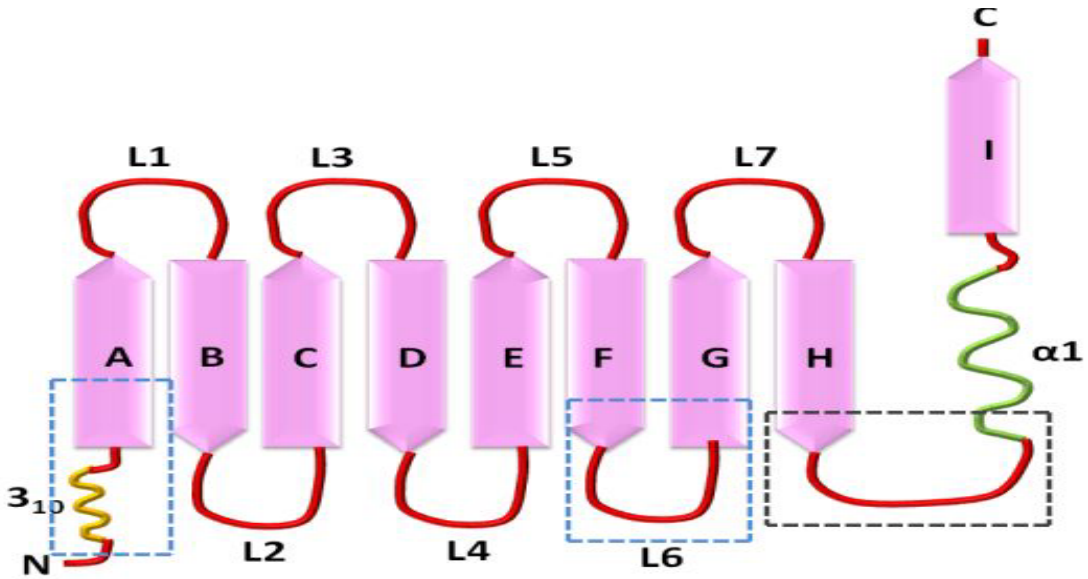
Source:"Acute Phase Proteins - Regulation and Functions of Acute Phase Proteins"book edited by Francisco Veas

NGAL has 198 aminoacids and is a glycoprotein and is coded by a genetic locus on chromosome 3p11.

At the N-terminal domain of NGAL is a 20 amino acid length signal peptide followed by the lipocalin domain. The lipocalin domain of NGAL is in the form of 8 anti parallel beta sheets in the form of a beta barrel[28].

There are three bulges seen in the secondary structure of NGAL. These form the site for binding of ligand for NGAL.The ligand binding site in lipocalin has hydrophobic residues like tryptophan, phenylalanine, valine. These residues are responsible for the direct binding of the NGAL to its ligands. These ligand binding residues are present in the base of the barrel[28].

Positively charged aminoacids like arginine and lysine present at the mouth of the barrel are also involved in ligand binding[28].



Source:"Acute Phase Proteins - Regulation and Functions of Acute Phase Proteins"book edited by Francisco Veas

NGAL is found in association with matrix metalloproteinase-9.At the base of the beta barrel, there is a negatively charged pit of glutamate and aspartate with a cysteine residue which is involved in binding with matrix metallo proteinase-9.Complex of NGAL with MMP-9 protect it from degradation.

NGAL can exist in any of the three forms:

1. Monomer –has weight of 25 kilo Dalton and is produced from the epithelial cells.
2. Homodimer-has weight of 45 kilo Dalton and is produced in the neutrophils.
3. Heterodimer-Found in association with MMP-9, has a weight of 135 kDa and is produced from epithelial cells. When MMP-9 is associated with NGAL, it prevents the auto degradation of MMP-9.

NGAL bound with its ligand is called holo- NGAL and its unbound form is called apo-NGAL[29].

NGAL is normally expressed in liver, kidney, adipose tissue, macrophages, thymus, prostate, small intestine, trachea, lungs. NGAL is found to be expressed in the fetal skin from the 20th week of gestation.

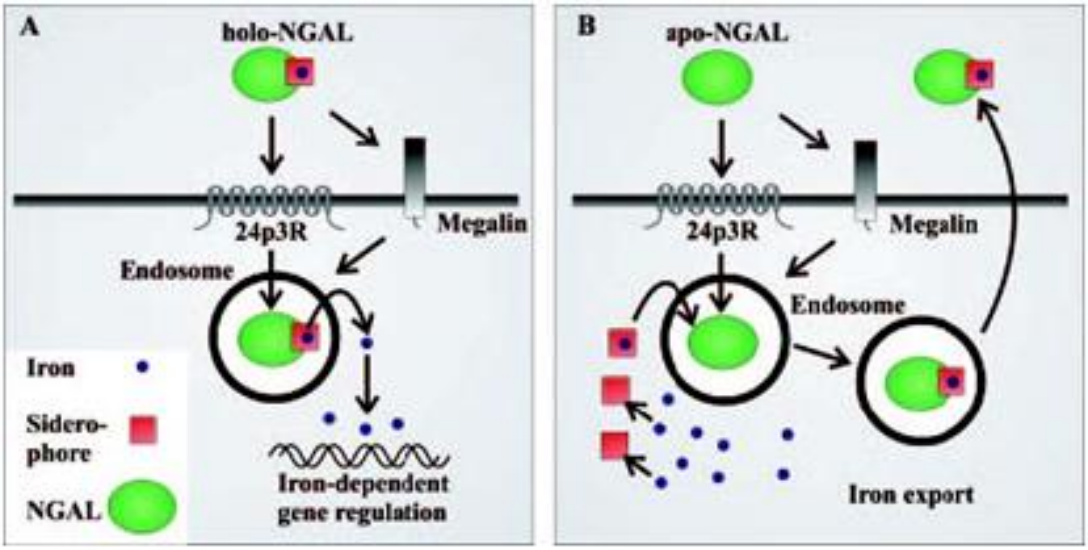
NGAL from the circulation is sieved by the glomerular capillaries and is again absorbed by the proximal convoluted tubule. It is also secreted in small amounts by thick ascending loop of henle. In the context of proximal tubular injury, there will be increased NGAL levels seen in serum and urine, due to the elevated production by the injured tubular cells as well as by the decreased tubular reabsorption.

NGAL has an important role in innate immunity against bacterial infections. Most bacteria require iron for their growth which they acquire from the host through iron binding molecules called siderophores. NGAL has high affinity for these siderophores and bind to them. This NGAL-siderophore complex is then taken into the cell through 24p3R receptor. This receptor has two transmembrane helices.

The NGAL-siderophore complex is then digested by the endosome. This results in the release of iron into the cytoplasm thus preventing the bacterial growth[30]. Thus NGAL has a physiological role as a bacteriostatic agent.

NGAL is related to physiological cell death in tissues of reproductive system. Epithelial cells of the involuting uterus and mammary glands present elevated levels of NGAL.NGAL is induced in response to apoptosis stressors and play a role in cell survival.

Research data imply that NGAL may be expressed by the damaged tubule to induce reepithelialisation and proliferation. During development, NGAL is expressed by the developing ureteric bud and stimulates nephrogenesis by triggering the change over of mesenchymal cells into epithelial cell types of kidney.



Source: Dual Action of Neutrophil Gelatinase–Associated Lipocalin, Kai M. Schmidt-Ott et al.J Am Soc Nephrol 18: 407–413, 2007. doi: 10.1681

In conditions of acute kidney injury, NGAL causes the leaning of the overall balance of proximal tubule cell fate is tilted towards cell longevity.

Conveyance of iron to the cells is vital for cell development and kidney regrowth after renal injury. As NGAL can be taken in by the proximal convoluted tubule, this peptide can recycle iron into the living cell. It may serve as a store house of iron that is liberated from the tubule cells that are impaired by renal injury.

Thus, apart from its anti-microbial action, NGAL is important in cell proliferation, apoptosis and differentiation by yet unidentified mechanisms. This occurs with the help of megalin-cubulin receptors in the proximal tubules of the kidney. In case of acute kidney injury there is up regulation of NGAL mRNA in the

proximal tubules and also in the distal tubules. This shows the tissue protective role of NGAL[30].

In view of the acute kidney injury associated with preeclampsia, there are few studies which were done to find out the usefulness of NGAL as a marker of preeclampsia.

Anna et al[31]., Burcu Artunc et al[32].,Youseff et al[33],Patel et al[15] have done independent case control studies and have shown that serum NGAL levels are much higher in preeclamptic patients than in their normotensive controls with statistical significance.

Grigorios et al[34] and Simonazzi et al[35] also have done case control studies in preeclamptic women and normotensive controls and they have suggested no statistically significant difference in the NGAL levels among the cases and controls.

MATERIALS AND METHODS

MATERIALS AND METHODS:

The study was conducted at PSG Institute of Medical Sciences and Research, Coimbatore. Ethical clearance was obtained from the Institutional Human Ethics Committee. An informed consent was taken from the patients before sample collection.

The study design is case control study in which pregnant women with preeclampsia (n =40) are selected as cases. The diagnosis of preeclampsia is based on case record description which is based on blood pressure measurements and urine protein creatinine ratio at the time of diagnosis. Cases were selected from pregnant women attending OG-OPD. Patients satisfying the diagnosis, inclusion criteria and not coming under exclusion criteria, were given explanation about this study. If they were willing to participate, the consent forms were filled and the samples were collected.

5ml of blood was collected in red topped vacutainers and was centrifuged at 3500 rpm for 10 minutes. The serum thus separated was aliquoted into smaller plain containers and stored at -20 degree Celsius for analysis. The urine sample was also collected.

Control individuals were also selected from the OG-OPD. Pregnant women fulfilling the control group criteria were requested to participate in the study. If they were willing, the consent forms were filled and samples were collected from them. Their samples were also processed and stored for analysis. Modified Kuppuswamy's scale was used for calculating the socioeconomic status of the patients.

INCLUSION CRITERIA:

Cases:

All pregnant women more than 20 weeks of gestation diagnosed with pre-eclampsia with blood pressure ≥ 140/90 mm of Hg or urine PCR ≥ 0.3

Controls:

Normal pregnant women more than 20 weeks of gestation without preeclampsia matched for gestational age.

EXCLUSION CRITERIA:

Subjects with

1. Known chronic hypertension
2. Known diabetes mellitus
3. Multiple pregnancy
4. Any other known medical disorder

Collection of blood samples:

For both cases and controls, blood was collected in red topped serum tube for the estimation of uric acid and urine was also collected for estimation of spot urine protein creatinine ratio. The left over serum after the estimation of uric acid was transferred to secondary labelled plain tube. This sample was stored at -20 degree celsius for NGAL assay.

The methods used for estimation of biochemical parameters were given below.

NGAL ELISA:

To quantify NGAL in serum by an enzyme immunoassay method

PRINCIPLE OF THE TEST:

This is a sandwich type immunoassay. The assay utilizes two monoclonal antibodies which are highly specific. The microtitre plate is precoated with an antibody specific to NGAL. Samples are then added to the appropriate microtiter plate wells with a biotin conjugated antibody specific to NGAL. Next avidin conjugated to horse radish peroxidase is added to each well and incubated. After tetra methyl benzidine (TMB) substrate solution is added, Only those wells containing NGAL, biotin conjugated antibody and enzyme conjugated avidin exhibit a change in colour. The enzyme substrate reaction is terminated by the addition of sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm using an ELISA reader.

PROCEDURE:

100 µl of sample per well is added and incubated for 2 hours.

The liquid is aspirated from each well(not washed).

100µl of detection reagent A is added and incubated for 1 hour.

Washed 3 times, detection reagent B is added and incubated for 30 minutes.

Washed 5 times and 90 µl of TMB substrate is added, incubated for 15 minutes.

50 µl of stop solution added. Read at 450 nm immediately.

SERUM URIC ACID:

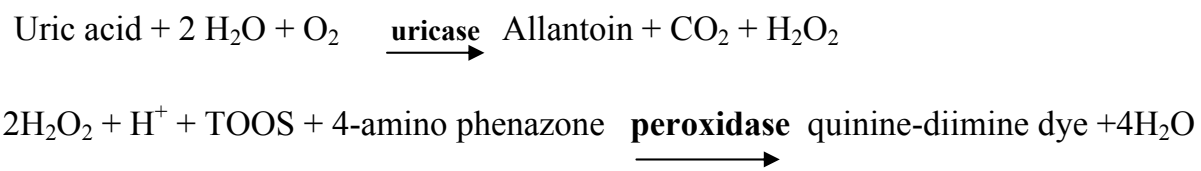
Method:

Enzymatic colorimetric test.

Principle:

Uricase cleaves uric acid to form allantoin and hydrogen peroxide. Hydrogen peroxide reacts with 4-aminophenazone to form quinine-diimine dye in the presence of peroxidase. The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance at 552 nm.

Reaction:



TOOS-N-ethyl-N-(2-hydroxy-3sulfopropyl)-3-methylaniline

Test definition:

Mode of measurement	Absorbance
Mode of calculation of absorbance	Endpoint
Mode of reaction	R1-S-SR
Direction of Reaction	Increase
Wavelength A/B	552 / 659 nm
Test value	0 – 1500 µmol/L (0 -25 mg/dL)
Unit	mg/dL

URINE PROTEIN CREATININE RATIO:

URINE PROTEIN:

Method:

Turbidimetric method.

Principle:

The sample is pre-incubated in an alkaline solution containing EDTA, which denatures the protein and eliminates interference from magnesium ions. Benzethonium chloride is then added, producing turbidity that is read at 512 nm.

Test definition:

Measuring Mode	Absorbance
Abs. calculation	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	512 nm
Test range	40-2000 mg/L (4-200 mg/dL)
with post dilution	40-6000 mg/L (4-600 mg/dL)
Unit	mg/dL

URINE CREATININE:

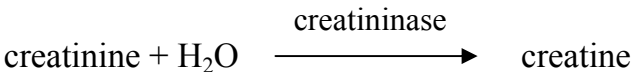
Method:

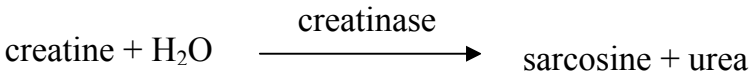
Enzymatic colorimetric method

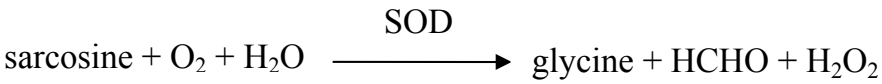
Principle:

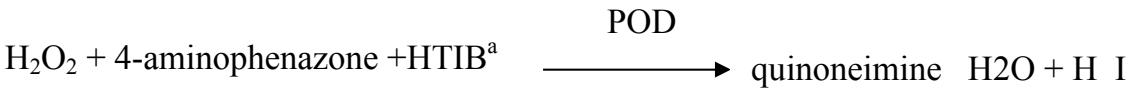
The enzymatic method is based on the established determination of hydrogen peroxide after conversion of creatinine with the aid of creatininase, creatinase and

sarcosine oxidase. The liberated hydrogen peroxide reacts with 4-aminophenazoneand HTIB to form a quinone imine chromogen.









(HTIB-2,4,6-triiodo-3-hydroxybenzoic acid)

The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration. It is determined by measuring the increase in absorbance at 552 nm.

Test definition:

Reaction Mode	D-R1- S-SR
Test range	0 – 40 mmol/L (0 – 452 mg/dL)
With Post dilution	0 - 200 mmol/L(0 – 2262 mg/dL)
Pre dilution factor	20
Post dilution factor	5 recommended
Unit	mg/dL

The ratio of urine protein to urine creatinine is calculated.

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS:

The data obtained were statistically analyzed using IBMSPSS software version19.

The data distribution was displayed by box and whisker plot and bar diagram. To find any statistically significant difference between the two groups in the distribution of the demographic characteristics and measured parameters, independent t-test was used. The dependence of categorical variable was tested with Chi Square test.

The correlation between quantitative parameters were done using Pearson correlation coefficient and the parameters with statistically significant correlations were further checked by scatter plot analysis.

RESULTS

RESULTS:

The distribution of demographic characteristics of the study population including maternal age, gestational age, body mass index, socio-economic status, blood group (mother, father, baby, mismatch between mother and baby) are given in figures 1,2,5,6,7,8,9,10 respectively. The distribution of some of these characteristics among cases and controls is represented in the form of box and whisker plots. In a box and whisker plot, only the inter-quartile range is taken into consideration and not the entire range, so the data more than 1.5 times the inter-quartile range is given as outliers in the distribution.

The characteristics like maternal age and gestational age are equally distributed in both the cases and controls showing that the two study groups are well matched for these parameters. The socioeconomic status of the two groups are also similar as shown by their distribution. The distribution of parameters like blood group of the mother, father, baby and mismatch between mother and baby's blood group are also not statistically significant between the two groups as indicated by chi square tests in the table-7,8,9,10.

The distribution of various other parameters including systolic blood pressure, diastolic blood pressure, serum uric acid, urine protein creatinine ratio are shown in figures 3,4,11,12. As expected, systolic blood pressure, diastolic blood pressure, serum uric acid level, urine protein creatinine ratio are higher in cases when compared with the control population.

The values of the t-test done to compare the means of these two groups' maternal age, gestational age, body mass index, socioeconomic status, serum uric acid, urine-PCR is shown in table 13. This also shows that there is a significant difference between the two groups with respect to parameters like systolic and diastolic blood pressure, body mass index, serum uric acid, urine PCR. It also shows that there is no significant difference between the two groups with regard to maternal age, gestational age of the mother at the time of sampling and socioeconomic status. This implies that the two groups are well matched in these parameters.

The serum NGAL level appears to be equally distributed in both the cases and controls as shown in figure-13.The t-test also does not show any significant difference in NGAL levels in these two groups as shown in table-14. There is no significant difference in NGAL level between the two groups.

The correlation coefficients between various parameters are shown in table 15. The corresponding scatter plots are shown in figure-14. The r values are significant for gestational age & urine PCR, BMI & urine PCR, uric acid & urine PCR, diastolic blood pressure& urine PCR, BMI & systolic blood pressure, BMI &diastolic blood pressure, diastolic blood pressure& uric acid, systolic blood pressure& uric acid, but only diastolic blood pressure & uric acid, systolic blood pressure& uric acid, uric acid & urine PCR showed positive linear relation in scatter plot.

Figure-1: Maternal age in study groups

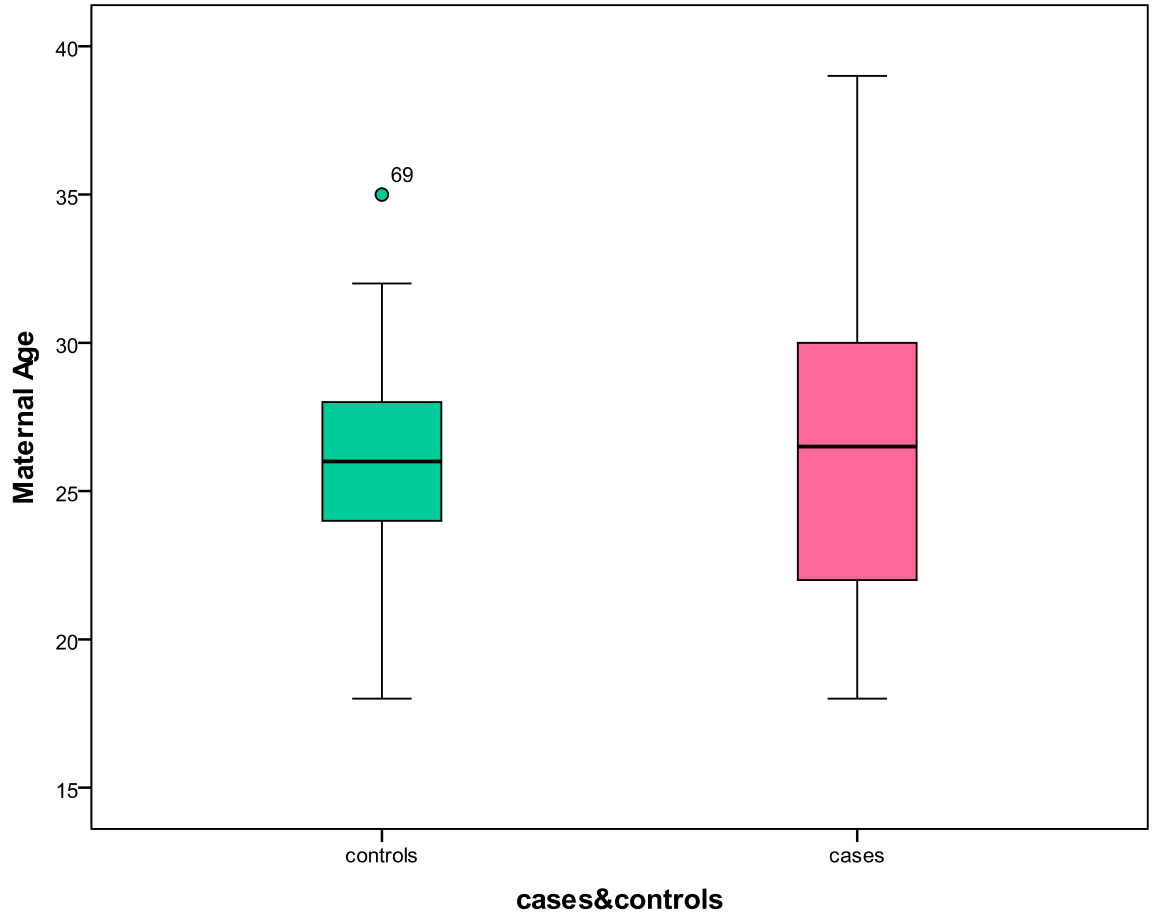


Table-1: Comparison of maternal age among study groups

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Maternal Age	Equal variances assumed	14.115	.000	-.663	78	.510	-.700	1.056
	Equal variances not assumed			-.663	62.614	.510	-.700	1.056

Figure-2: Gestational age in study groups

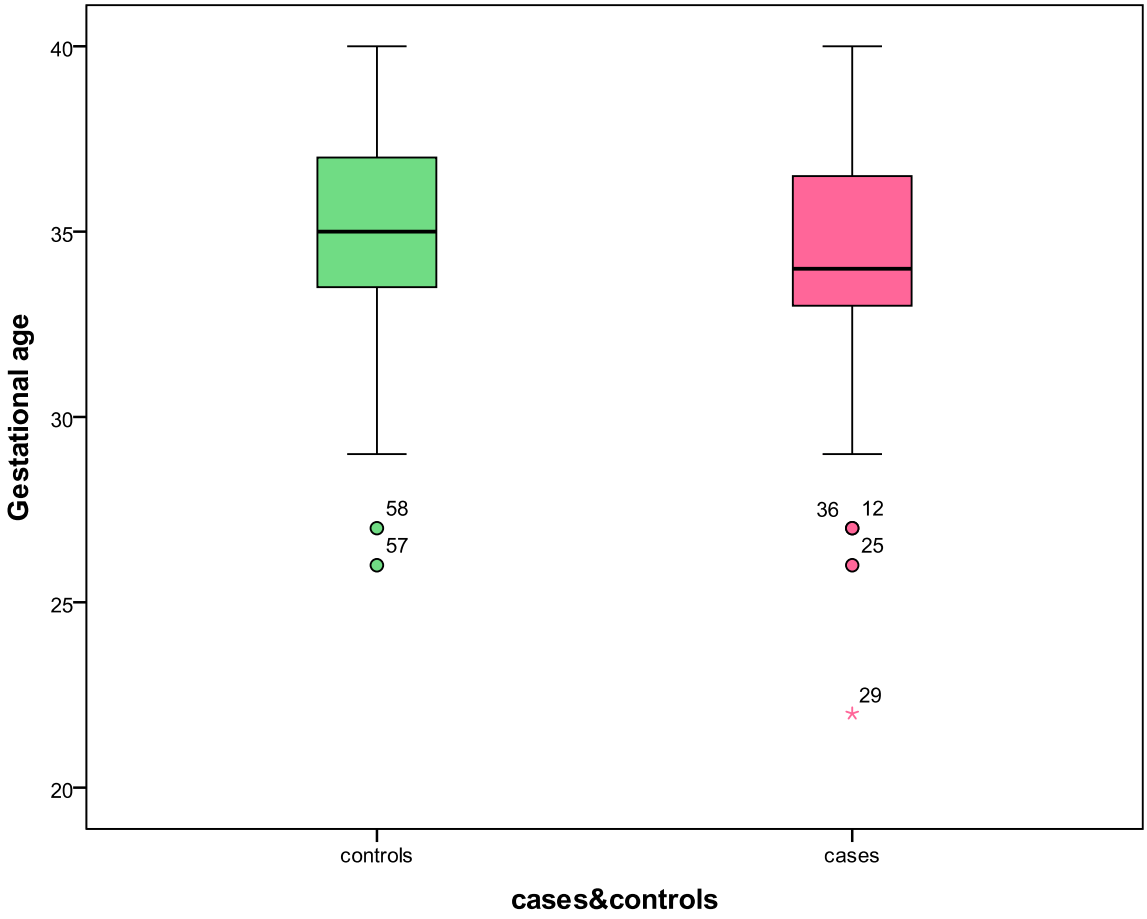


Table-2:Comparison of gestational age among study groups

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Gestational age	Equal variances assumed	.412	.523	.930	78	.355	.725	.780	-.828	2.278
	Equal variances not assumed			.930	74.327	.356	.725	.780	-.829	2.279

Figure-3: Systolic Blood pressure in study groups

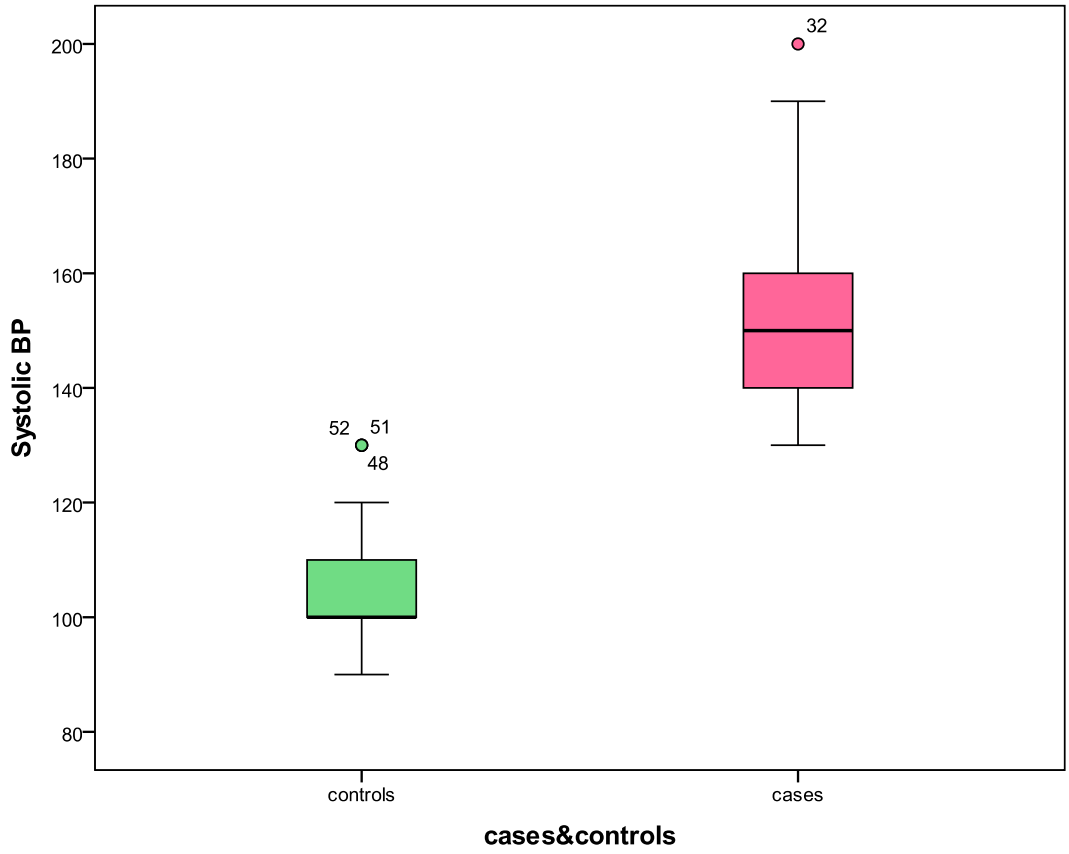


Table-3:Comparison of systolic blood pressure in study groups

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Systolic BP	Equal variances assumed	4.881	.030	-15.739	78	.000	-49.625	3.153	-55.902	-43.348
	Equal variances not assumed			-15.739	69.648	.000	-49.625	3.153	-55.914	-43.336

Figure-4:Diastolic Blood pressure in study groups

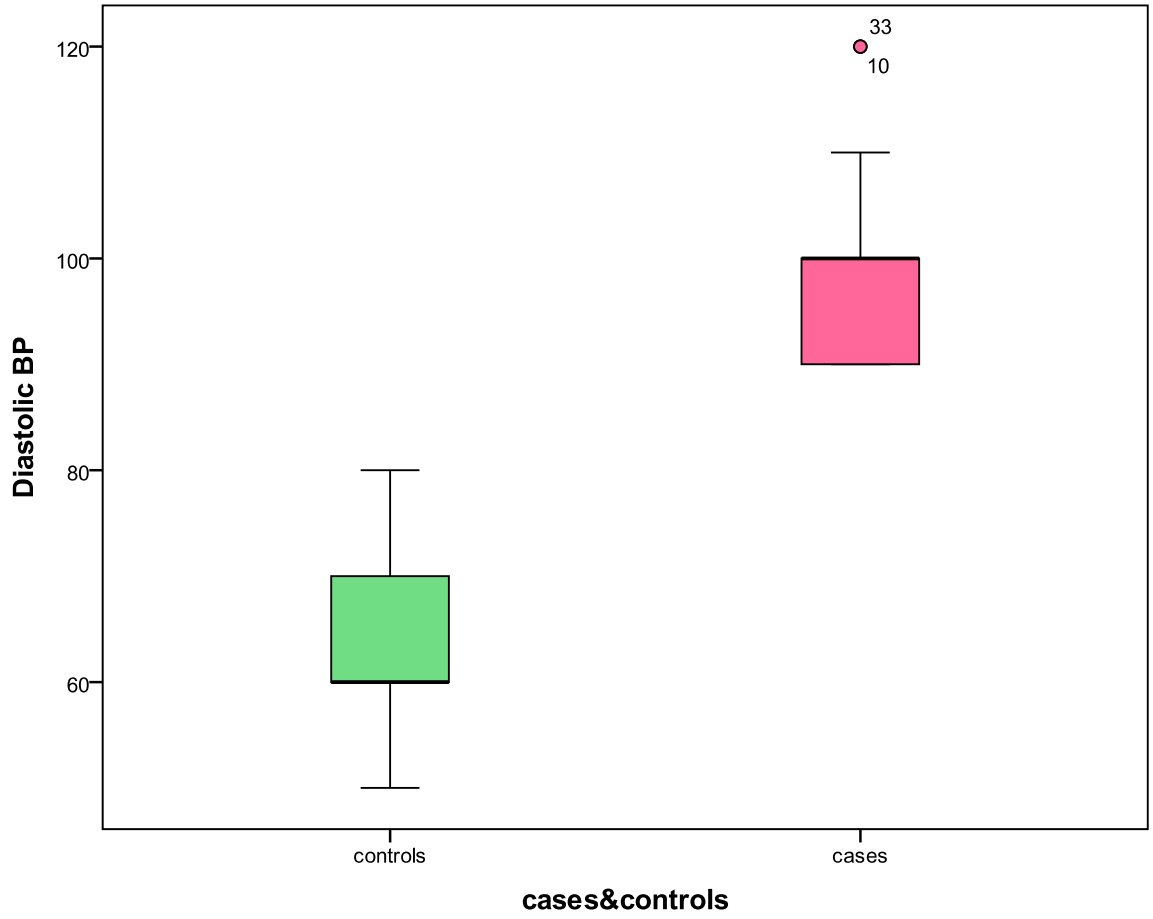


Table-4: Comparison of diastolic blood pressure in study groups

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Diastolic BP	Equal variances assumed	2.281	.135	-18.629	78	.000	-33.000	1.771	-36.527	-29.473
	Equal variances not assumed			-18.629	72.861	.000	-33.000	1.771	-36.530	-29.470

Figure-5:Body Mass Index in study groups

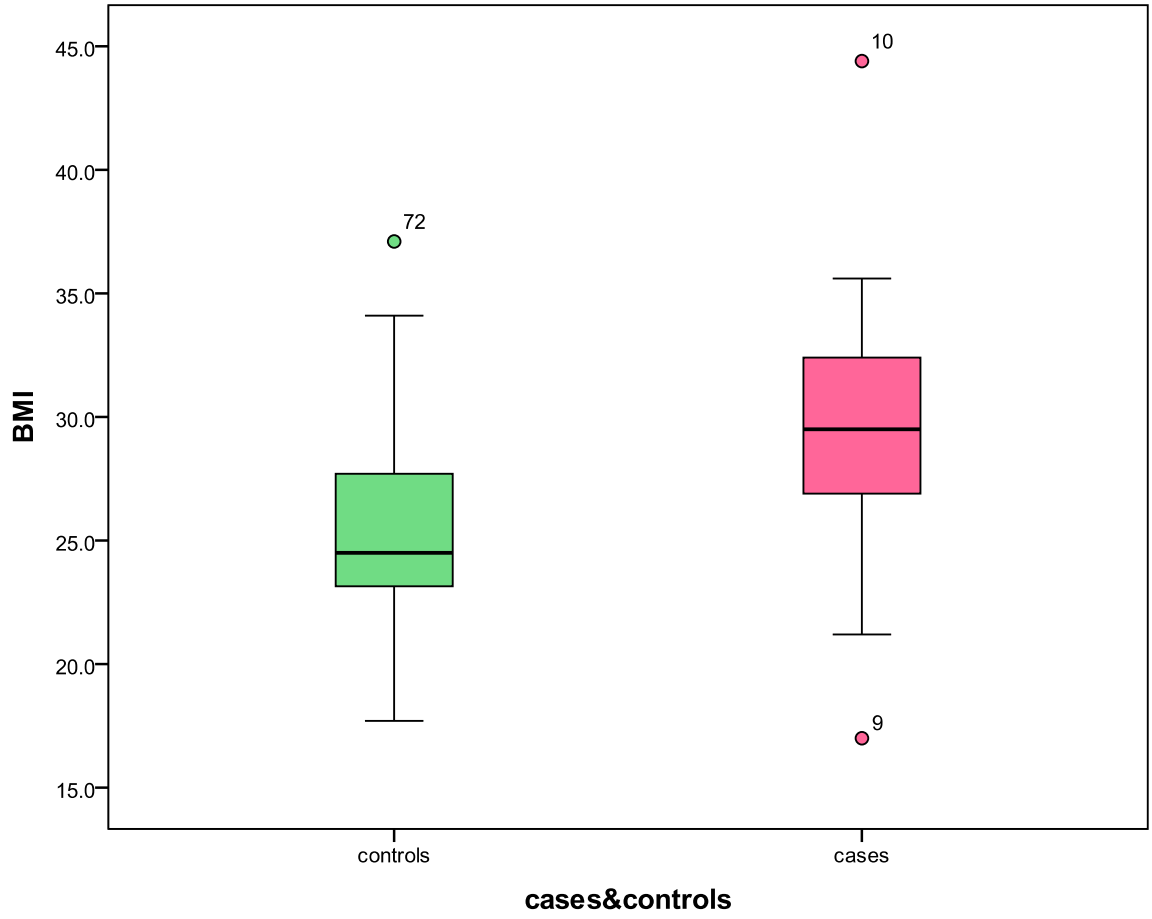


Table-5: Comparison of body mass index in study groups

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
BMI	Equal variances assumed	.256	.615	-3.462	78	.001	-3.5890	1.0367	-5.6528	-1.5252
	Equal variances not assumed			-3.462	76.429	.001	-3.5890	1.0367	-5.6535	-1.5245

Figure-6:Distribution of socio-economic status in study groups:

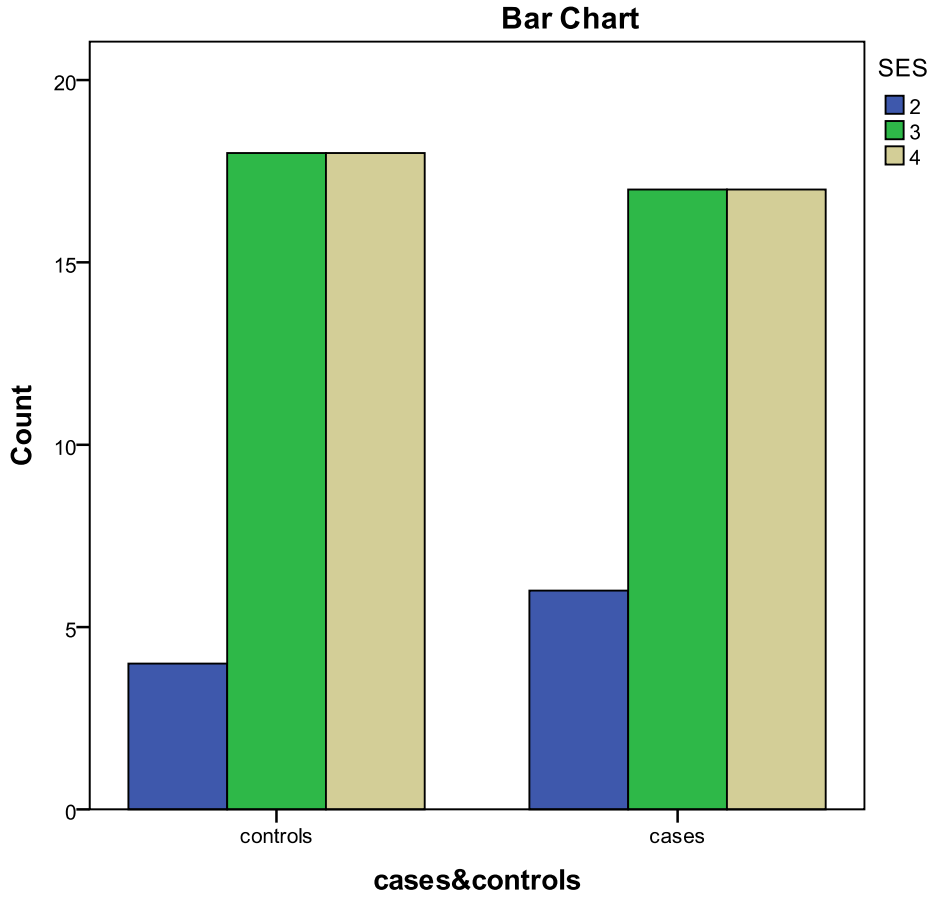


Table-6-Comparison of socio-economic status in study groups:

cases&controls * SES Crosstabulation

Count

		SES			Total
		2	3	4	
cases&controls	controls	4	18	18	40
	cases	6	17	17	40
Total		10	35	35	80

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.457[a]	2	.796
Likelihood Ratio	.460	2	.795
Linear-by-Linear Association	.239	1	.625
N of Valid Cases	80		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.00.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Gamma	-.083	.194	-.426	670
Measure of Agreement	Kappa	.000	.000	.	
N of Valid Cases		80			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Figure-7:Distribution of mother's blood group in study groups:

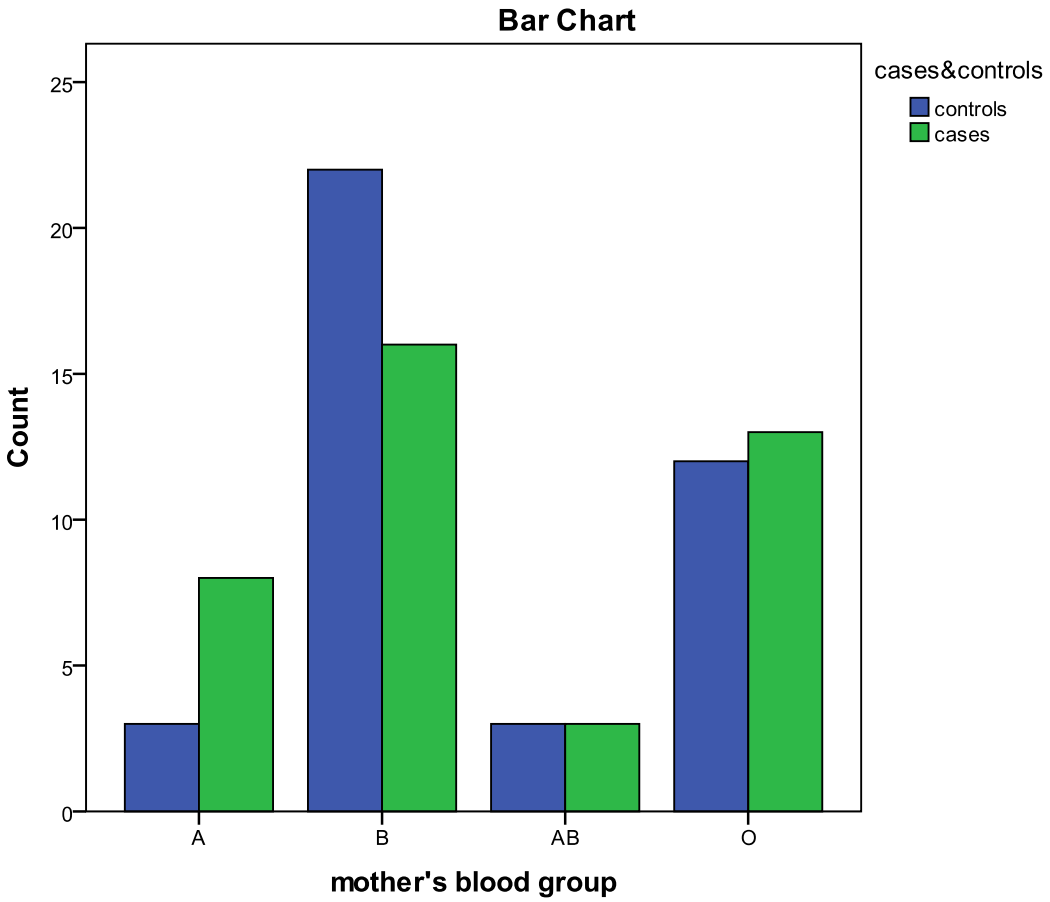


Table-7 :Comparison of mother's blood group in study groups

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.260[a]	3	.353
Likelihood Ratio	3.350	3	.341
Linear-by-Linear Association	.097	1	.756
N of Valid Cases	80		

Mother's blood group * cases&controls Crosstabulation

			cases&controls		
			controls	cases	Total
mother's blood group	A	Count	3	8	11
		Expected Count	5.5	5.5	11.0
		% within mother's blood group	27.3%	72.7%	100.0%
		% within cases&controls	7.5%	20.0%	13.8%
		% of Total	3.8%	10.0%	13.8%
	B	Count	22	16	38
		Expected Count	19.0	19.0	38.0
		% within mother's blood group	57.9%	42.1%	100.0%
		% within cases&controls	55.0%	40.0%	47.5%
		% of Total	27.5%	20.0%	47.5%
	AB	Count	3	3	6
		Expected Count	3.0	3.0	6.0
		% within mother's blood group	50.0%	50.0%	100.0%
		% within cases&controls	7.5%	7.5%	7.5%
		% of Total	3.8%	3.8%	7.5%
	O	Count	12	13	25
		Expected Count	12.5	12.5	25.0
		% within mother's blood group	48.0%	52.0%	100.0%
		% within cases&controls	30.0%	32.5%	31.3%
		% of Total	15.0%	16.3%	31.3%
Total		Count	40	40	80
		Expected Count	40.0	40.0	80.0
		% within mother's blood group	50.0%	50.0%	100.0%
		% within cases&controls	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Figure-8:Distribution of father's blood group in study groups:

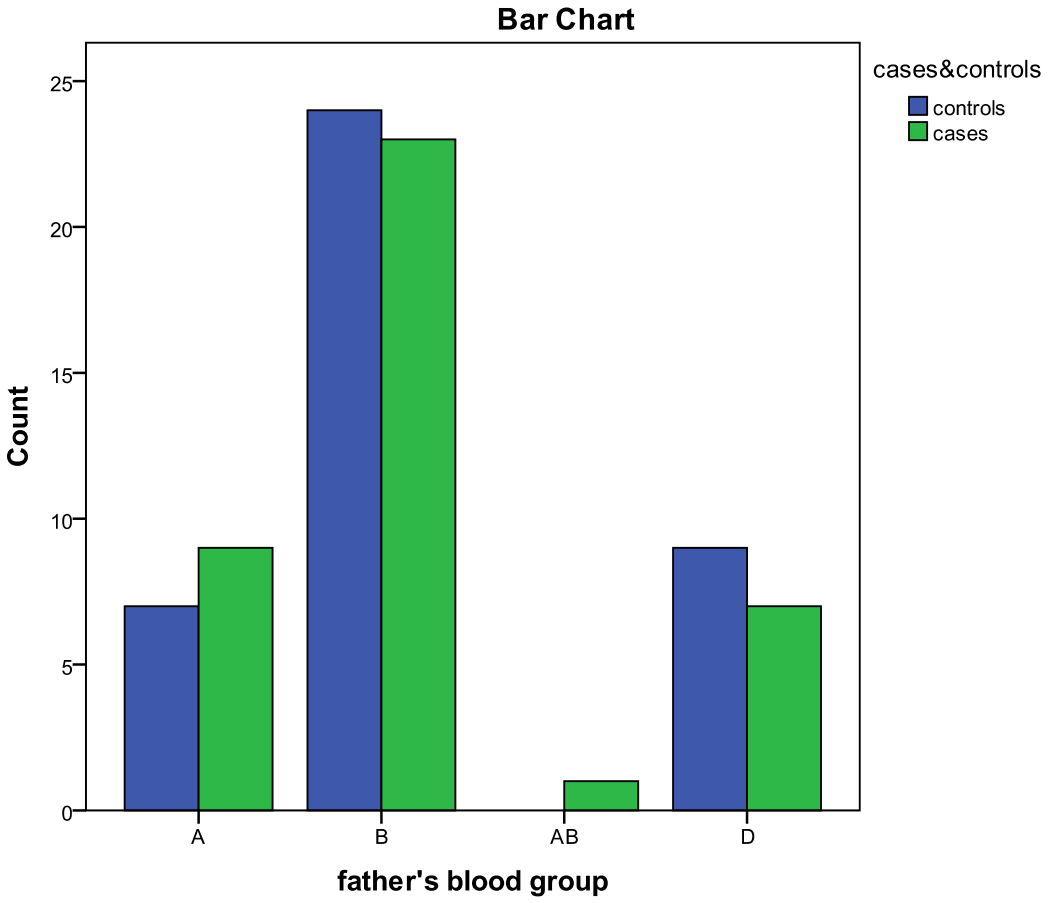


Table-8:Comparison of father's blood group in study groups

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.521[a]	3	.677
Likelihood Ratio	1.909	3	.592
Linear-by-Linear Association	.319	1	.572
N of Valid Cases	80		

a. 2 cells (25.0%) have expected count less than 5. The minimum expected count is .50.

Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.521[a]	3	.677
Likelihood Ratio	1.909	3	.592
Linear-by-Linear Association	.319	1	.572
N of Valid Cases	80		

Father's blood group * cases&controls Crosstabulation

			cases&controls		
			controls	cases	Total
father's blood group	A	Count	7	9	16
		Expected Count	8.0	8.0	16.0
		% within father's blood group	43.8%	56.3%	100.0%
		% within cases&controls	17.5%	22.5%	20.0%
		% of Total	8.8%	11.3%	20.0%
	B	Count	24	23	47
		Expected Count	23.5	23.5	47.0
		% within father's blood group	51.1%	48.9%	100.0%
		% within cases&controls	60.0%	57.5%	58.8%
		% of Total	30.0%	28.8%	58.8%
	AB	Count	0	1	1
		Expected Count	.5	.5	1.0
		% within father's blood group	.0%	100.0%	100.0%
		% within cases&controls	.0%	2.5%	1.3%
		% of Total	.0%	1.3%	1.3%
	O	Count	9	7	16
		Expected Count	8.0	8.0	16.0
		% within father's blood group	56.3%	43.8%	100.0%
		% within cases&controls	22.5%	17.5%	20.0%
		% of Total	11.3%	8.8%	20.0%
Total		Count	40	40	80
		Expected Count	40.0	40.0	80.0
		% within father's blood group	50.0%	50.0%	100.0%
		% within cases&controls	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Figure-9:Distribution ofbaby's blood group in study groups:

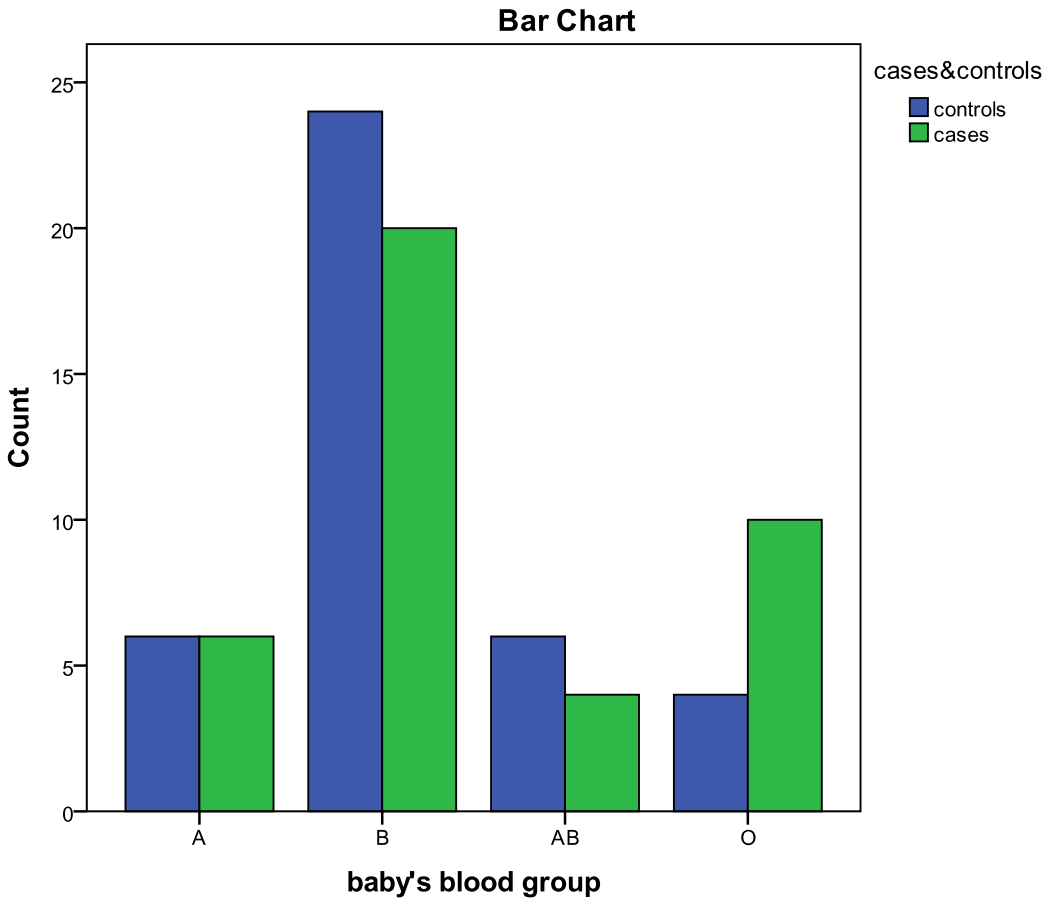


Table-9: Comparison of baby's blood group in study groups

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.335[a]	3	.343
Likelihood Ratio	3.423	3	.331
Linear-by-Linear Association	1.420	1	.233
N of Valid Cases	80		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 5.00.

Baby's blood group * cases &controls Crosstabulation

			cases&controls		
			controls	cases	Total
baby's blood group	A	Count	6	6	12
		Expected Count	6.0	6.0	12.0
		% within baby's blood group	50.0%	50.0%	100.0%
		% within cases&controls	15.0%	15.0%	15.0%
		% of Total	7.5%	7.5%	15.0%
	B	Count	24	20	44
		Expected Count	22.0	22.0	44.0
		% within baby's blood group	54.5%	45.5%	100.0%
		% within cases&controls	60.0%	50.0%	55.0%
		% of Total	30.0%	25.0%	55.0%
	AB	Count	6	4	10
		Expected Count	5.0	5.0	10.0
		% within baby's blood group	60.0%	40.0%	100.0%
		% within cases&controls	15.0%	10.0%	12.5%
		% of Total	7.5%	5.0%	12.5%
	O	Count	4	10	14
		Expected Count	7.0	7.0	14.0
		% within baby's blood group	28.6%	71.4%	100.0%
		% within cases&controls	10.0%	25.0%	17.5%
		% of Total	5.0%	12.5%	17.5%
Total		Count	40	40	80
		Expected Count	40.0	40.0	80.0
		% within baby's blood group	50.0%	50.0%	100.0%
		% within cases&controls	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Figure-10:Distribution ofmother-baby blood group mismatch in study groups:

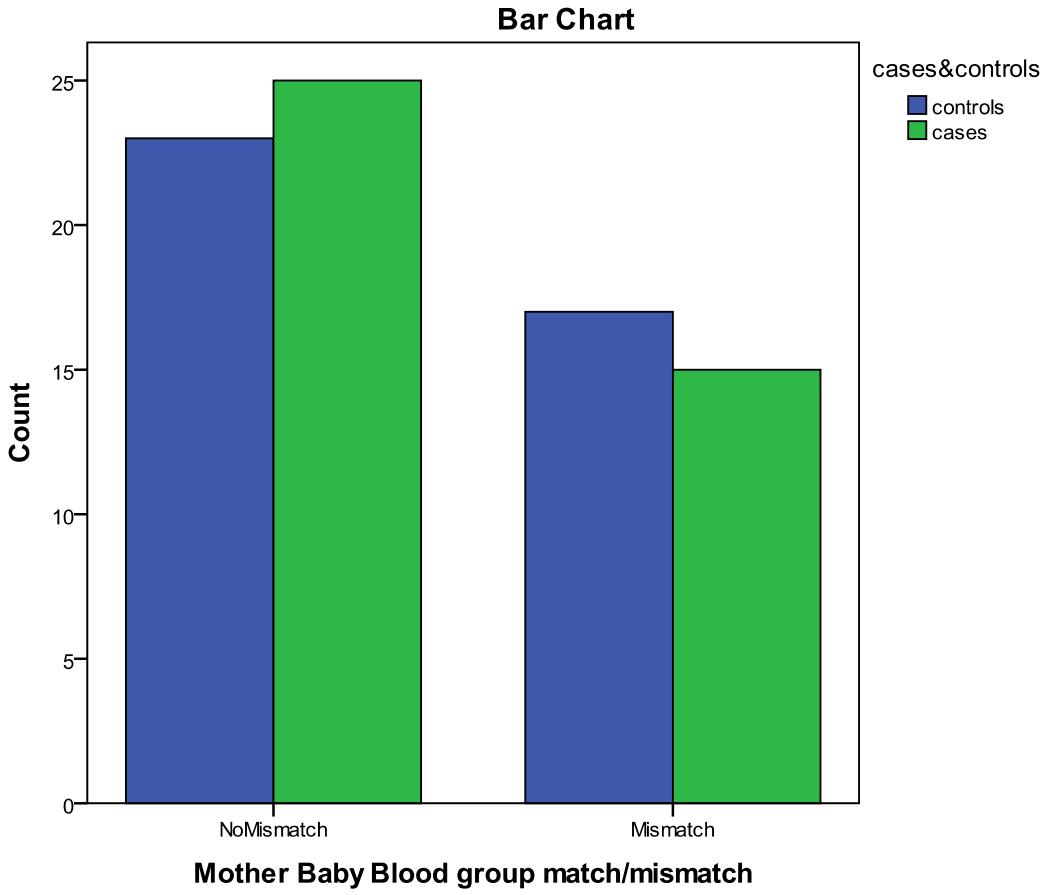


Table-10 :Comparison ofmother-baby blood group mismatch in study groups:

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.208[a]	1	.648		
Continuity Correction[b]	.052	1	.819		
Likelihood Ratio	.208	1	.648		
Fisher's Exact Test				.820	.410
N of Valid Cases	80				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16.00.

b. Computed only for a 2x2 table

Mother Baby Blood group match/mismatch * cases & controls Cross tabulation

			cases&controls		
			controls	cases	Total
Mother Baby Blood group match/mismatch	No Mismatch	Count	23	25	48
		Expected Count	24.0	24.0	48.0
		% within Mother Baby Blood group match/mismatch	47.9%	52.1%	100.0%
		% within cases&controls	57.5%	62.5%	60.0%
		% of Total	28.8%	31.3%	60.0%
	Mismatch	Count	17	15	32
		Expected Count	16.0	16.0	32.0
		% within Mother Baby Blood group match/mismatch	53.1%	46.9%	100.0%
		% within cases&controls	42.5%	37.5%	40.0%
		% of Total	21.3%	18.8%	40.0%
Total		Count	40	40	80
		Expected Count	40.0	40.0	80.0
		% within Mother Baby Blood group match/mismatch	50.0%	50.0%	100.0%
		% within cases&controls	100.0%	100.0%	100.0%
		% of Total	50.0%	50.0%	100.0%

Figure-11:Serum Uric acid level in study groups

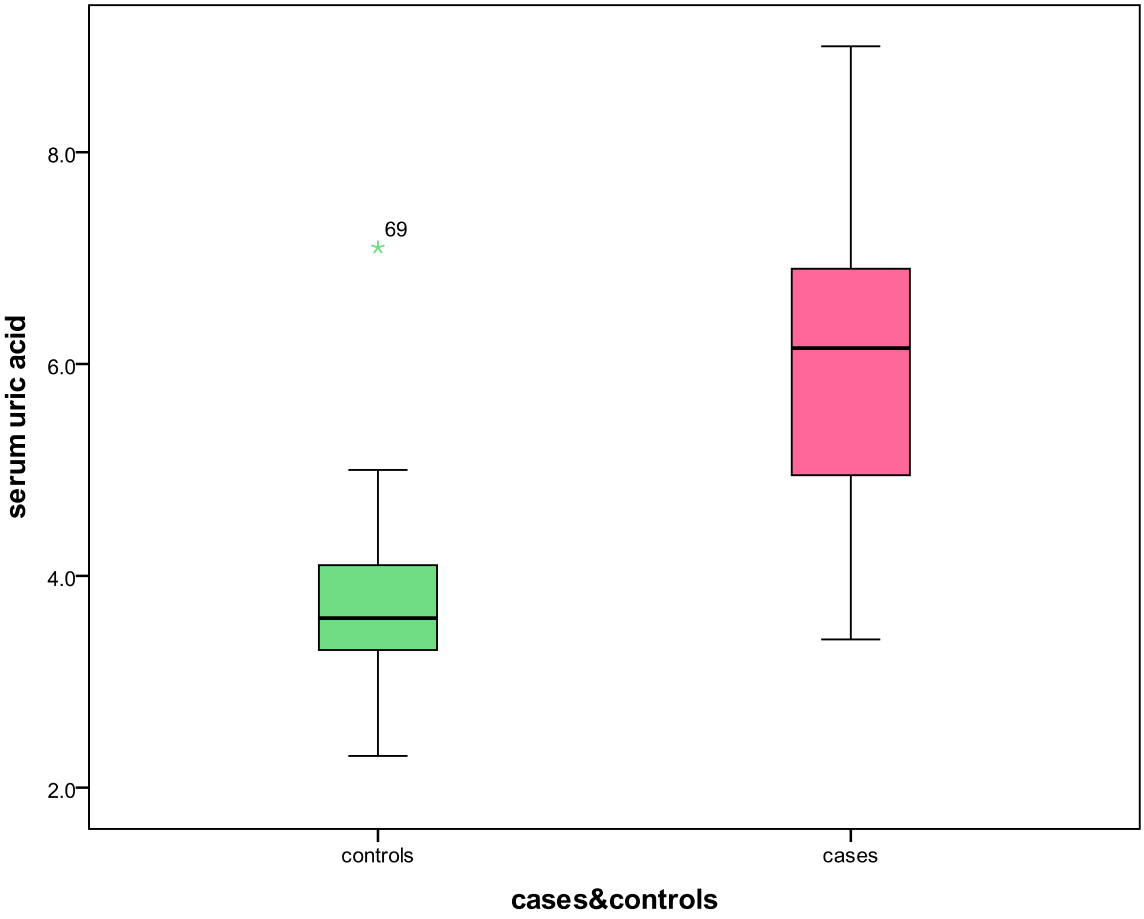


Table-11: Comparison of uric acid level in study groups

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
BMI	Equal variances assumed	.256	.615	-3.462	78	.001	-3.5890	1.0367	-5.6528	-1.5252
	Equal variances not assumed			-3.462	76.429	.001	-3.5890	1.0367	-5.6535	-1.5245

Figure-12:Urine Protein –creatinine ratio in study groups

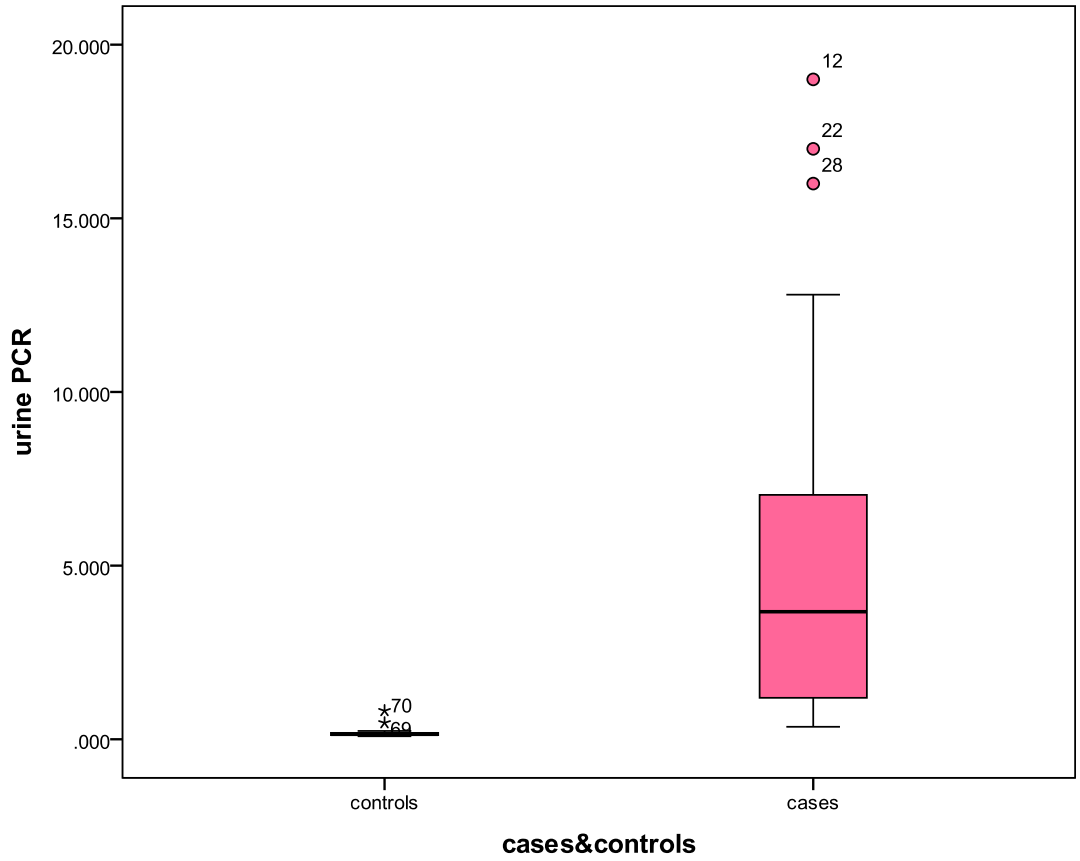


Table-12: Comparison of urine PCR in study groups

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
urine PCR	Equal variances assumed	58.764	.000	-6.273	78	.000	-4.893150	.780071	-6.446153	-3.340147
	Equal variances not assumed			-6.273	39.050	.000	-4.893150	.780071	-6.470929	-3.315371

Table-13: t-test values for comparison of parameters between the two groups

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Maternal Age	Equal variances assumed	14.115	.000	-.663	78	.510	-.700	1.056	-2.803	1.403
	Equal variances not assumed			-.663	62.614	.510	-.700	1.056	-2.811	1.411
BMI	Equal variances assumed	.256	.615	-3.462	78	.001	-3.5890	1.0367	-5.6528	-1.5252
	Equal variances not assumed			-3.462	76.429	.001	-3.5890	1.0367	-5.6535	-1.5245
gest. age	Equal variances assumed	.412	.523	.930	78	.355	.725	.780	-.828	2.278
	Equal variances not assumed			.930	74.327	.356	.725	.780	-.829	2.279
Systolicc BP	Equal variances assumed	4.881	.030	-15.739	78	.000	-49.625	3.153	-55.902	-43.348
	Equal variances not assumed			-15.739	69.648	.000	-49.625	3.153	-55.914	-43.336
Diastolic BP	Equal variances assumed	2.281	.135	-18.629	78	.000	-33.000	1.771	-36.527	-29.473
	Equal variances not assumed			-18.629	72.861	.000	-33.000	1.771	-36.530	-29.470
serum uric acid	Equal variances assumed	13.011	.001	-8.700	78	.000	-2.4200	.2781	-2.9738	-1.8662
	Equal variances not assumed			-8.700	60.126	.000	-2.4200	.2781	-2.9764	-1.8636
urine PCR	Equal variances assumed	58.764	.000	-6.273	78	.000	-4.893150	.780071	-6.446153	-3.340147
	Equal variances not assumed			-6.273	39.050	.000	-4.893150	.780071	-6.470929	-3.315371

Figure-13: Serum NGAL levels in study groups

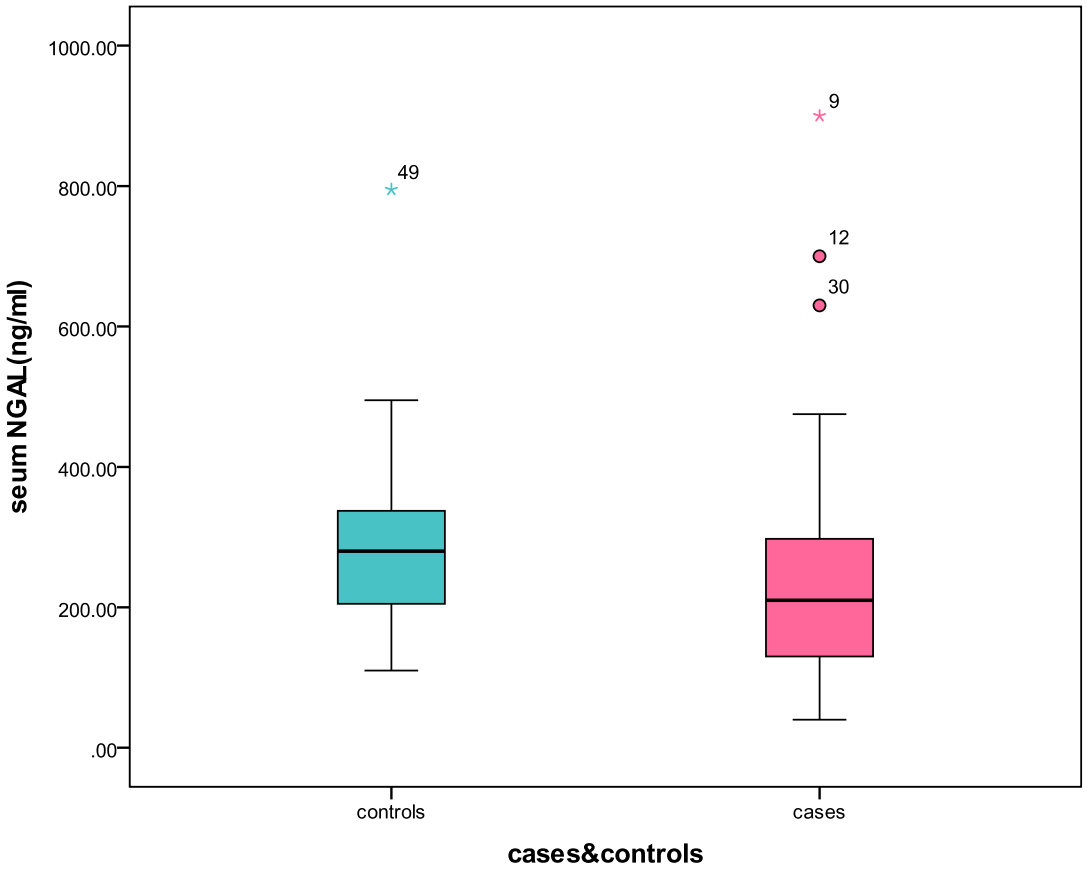
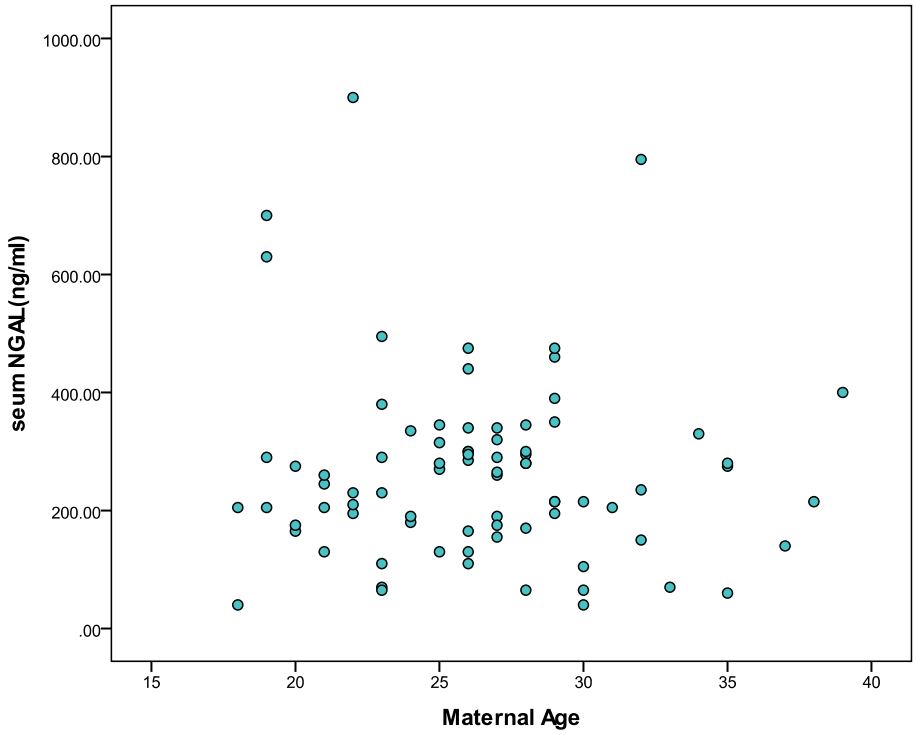


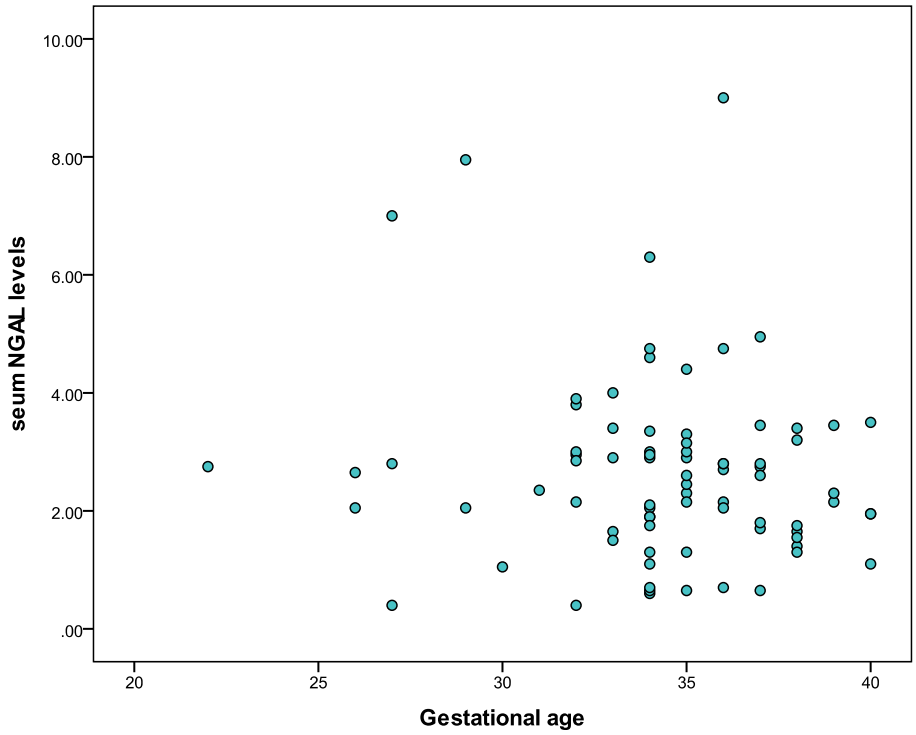
Table-14: Serum NGAL levels in study groups

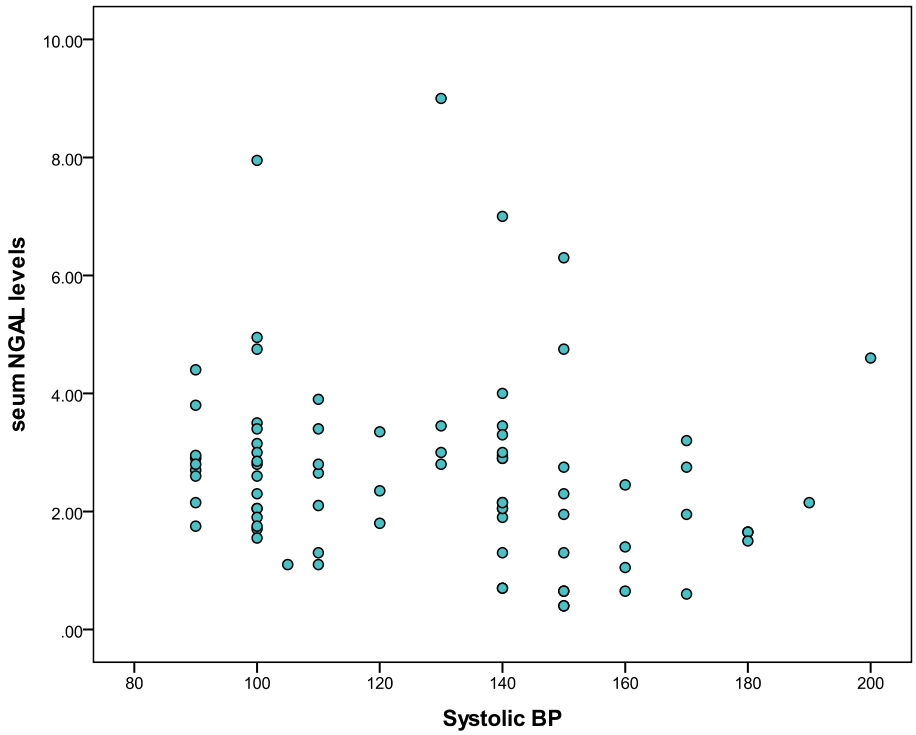
Independent Samples Test

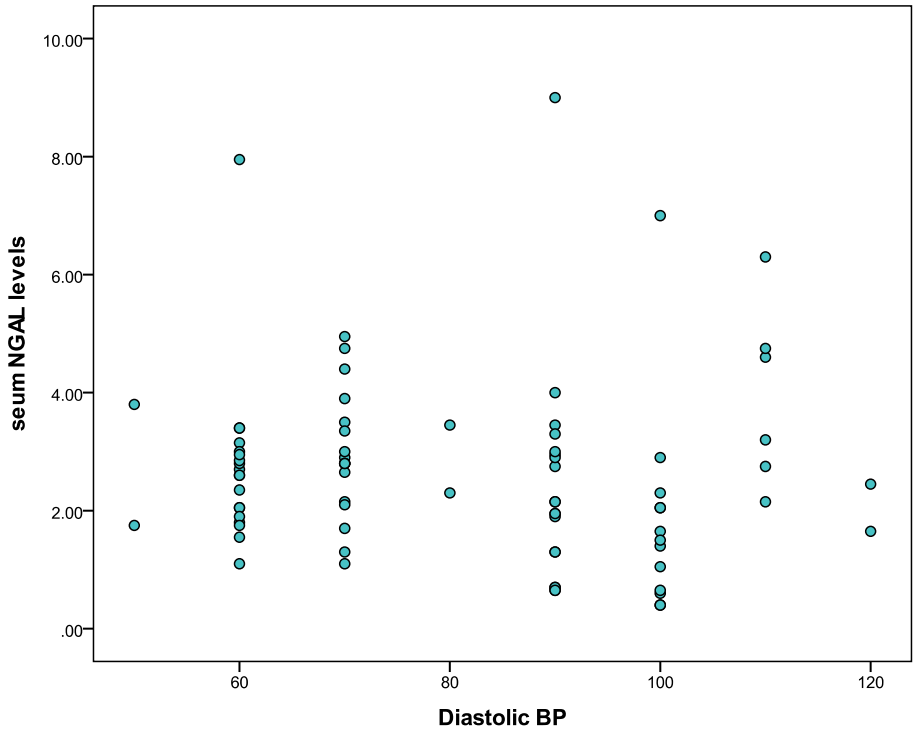
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
seum NGAL levels	Equal variances assumed	3.577	.062	1.063	78	.291	.37125	.34935	-.32426	1.06676
	Equal variances not assumed			1.063	68.271	.292	.37125	.34935	-.32583	1.06833

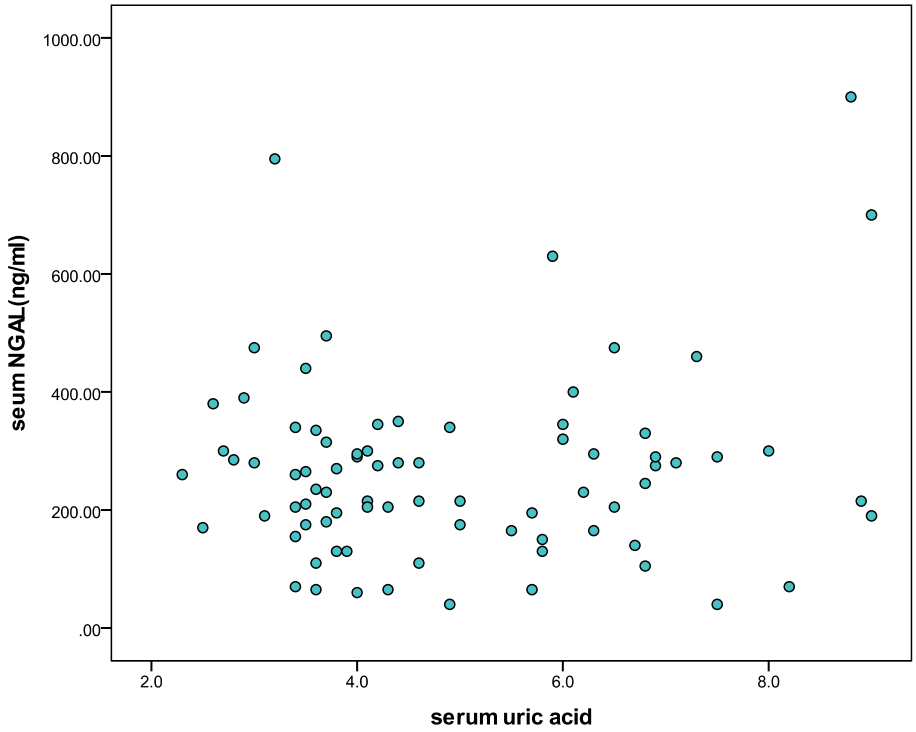
FIGURE-14:SCATTER PLOTS OF NGAL WITH VARIOUS PARAMETERS






10.00
8.00
6.00
4.00
2.00
.00
seum NGAL levels
80
100
120
140
160
180
200
Systolic BP


10.00
8.00
6.00
4.00
2.00
.00
seum NGAL levels
60
80
100
120
Diastolic BP


1000.00
800.00
600.00
400.00
200.00
.00
seum NGAL(ng/ml)
2.0
4.0
6.0
8.0
serum uric acid

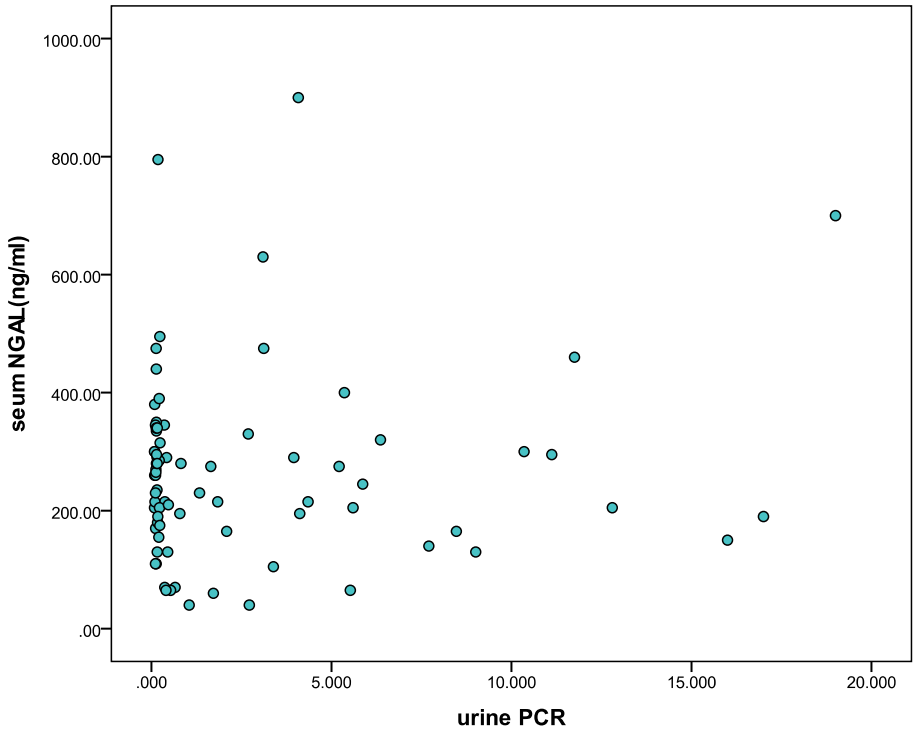

1000.00
800.00
600.00
400.00
200.00
.00
seum NGAL(ng/ml)
.000
5.000
10.000
15.000
20.000
urine PCR

Table-15- Correlation coefficient between various parameters:

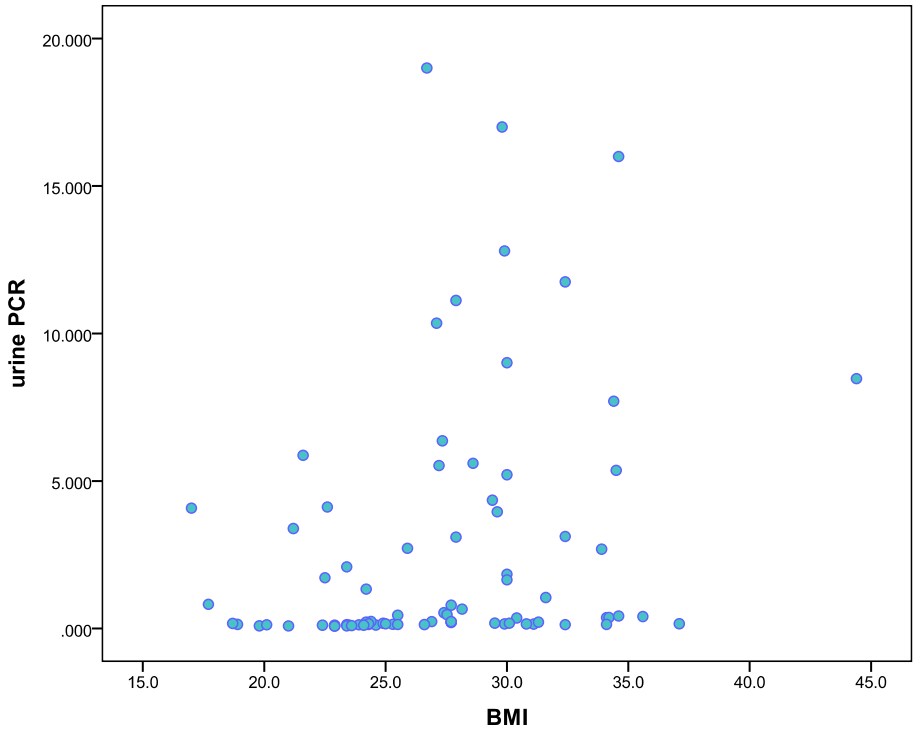
Correlations

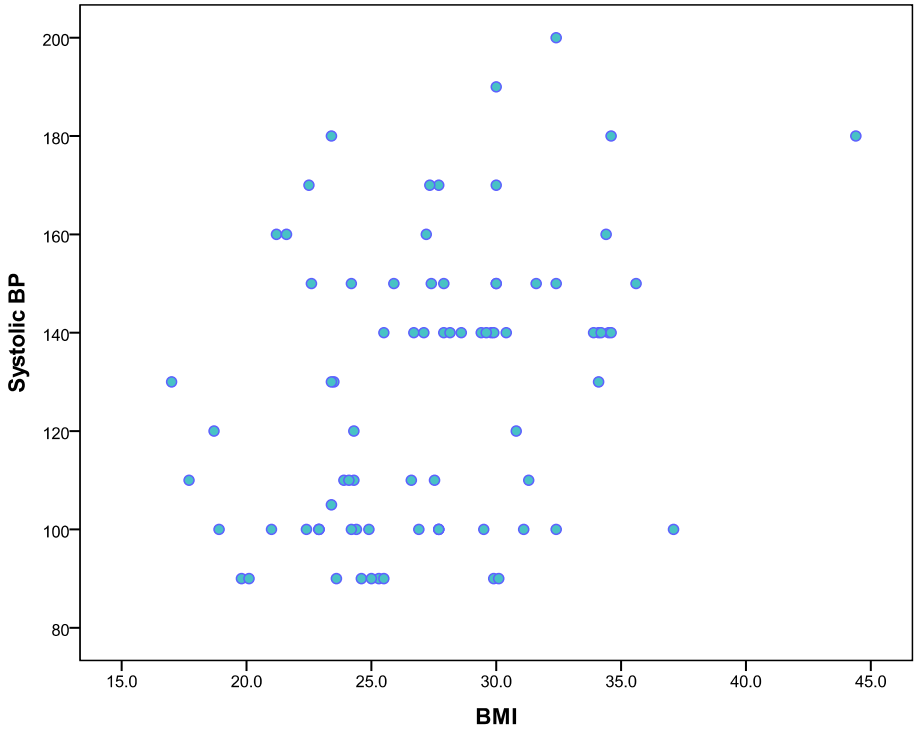
		Maternal Age	BMI	Systolic BP	Diastolic BP	gest.age	serum uric acid	urine PCR	seum NGAL levels
Maternal Age		1	.172	.130	.065	-.071	-.060	.002	-.073
			.127	.250	.568	.534	.597	.987	.522
		80	80	80	80	80	80	80	80
BMI		.172	1	.362**	.339**	-.031	.189	.268*	-.076
		.127		.001	.002	.787	.093	.016	.502
		80	80	80	80	80	80	80	80
SystolicBP		.130	.362**	1	.902**	-.084	.549**	.539**	-.176
		.250	.001		.000	.460	.000	.000	.119
		80	80	80	80	80	80	80	80
Diastolic BP		.065	.339**	.902**	1	-.127	.628**	.575**	-.066
	Sig. (2-tailed)	.568	.002	.000		.263	.000	.000	.563
	N	80	80	80	80	80	80	80	80
gest.age	Pearson Correlation	-.071	-.031	-.084	-.127	1	-.085	-.274*	-.117
	Sig. (2-tailed)	.534	.787	.460	.263		.453	.014	.300
	N	80	80	80	80	80	80	80	80
serum uric acid	Pearson Correlation	-.060	.189	.549**	.628**	-.085	1	.626**	.129
	Sig. (2-tailed)	.597	.093	.000	.000	.453		.000	.254
	N	80	80	80	80	80	80	80	80
urine PCR	Pearson Correlation	.002	.268*	.539**	.575**	-.274*	.626**	1	.107
	Sig. (2-tailed)	.987	.016	.000	.000	.014	.000		.343
	N	80	80	80	80	80	80	80	80
seum NGAL levels	Pearson Correlation	-.073	-.076	-.176	-.066	-.117	.129	.107	1
	Sig. (2-tailed)	.522	.502	.119	.563	.300	.254	.343	
	N	80	80	80	80	80	80	80	80

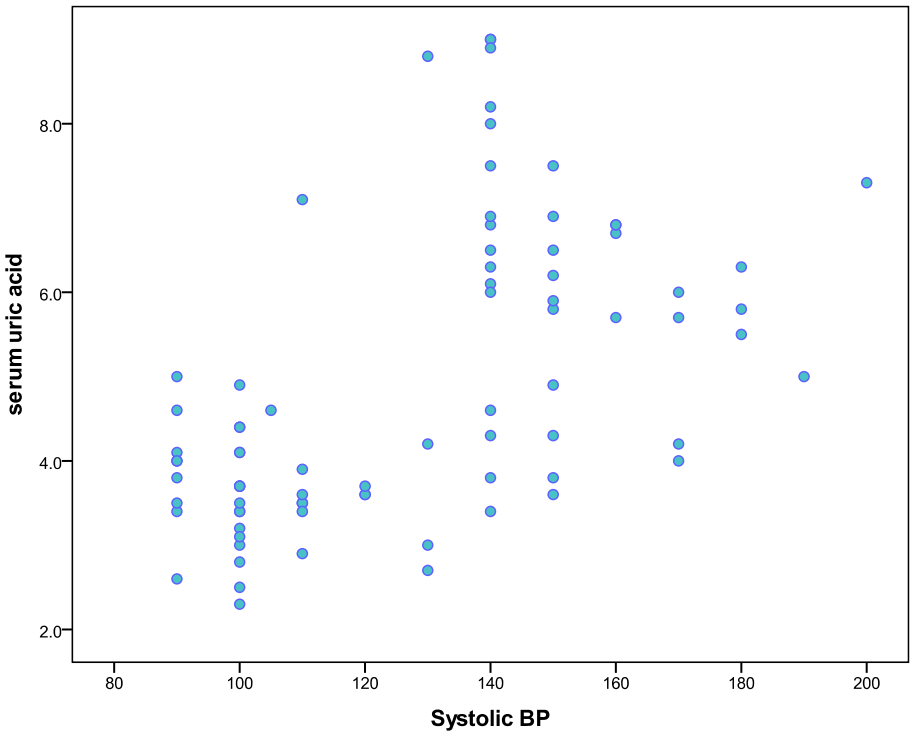
**. Correlation is significant at the 0.01 level (2-tailed).

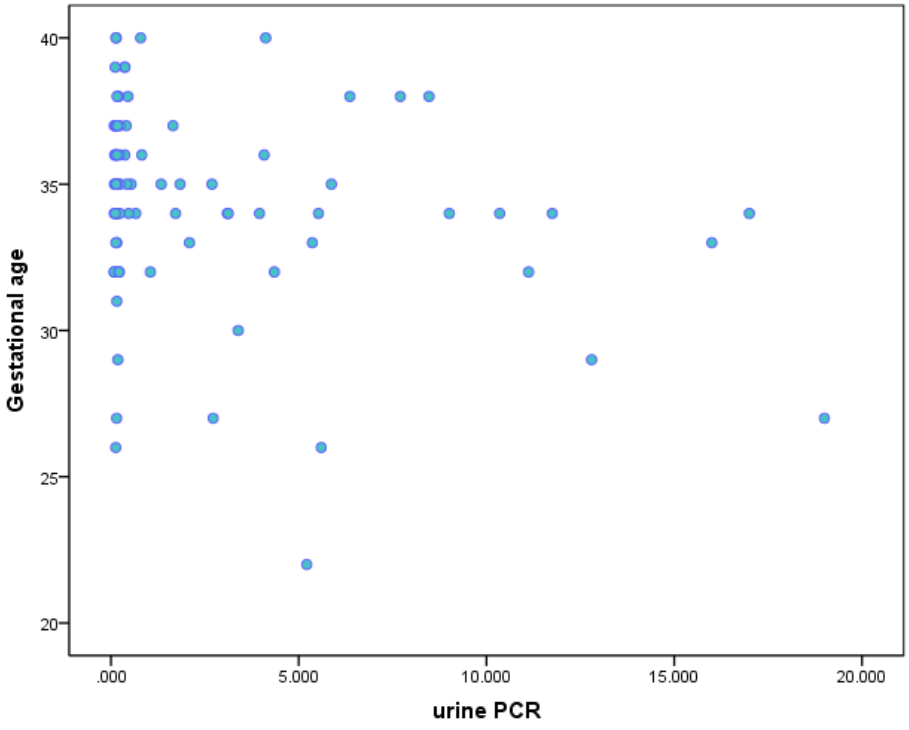
*. Correlation is significant at the 0.05 level (2-tailed).

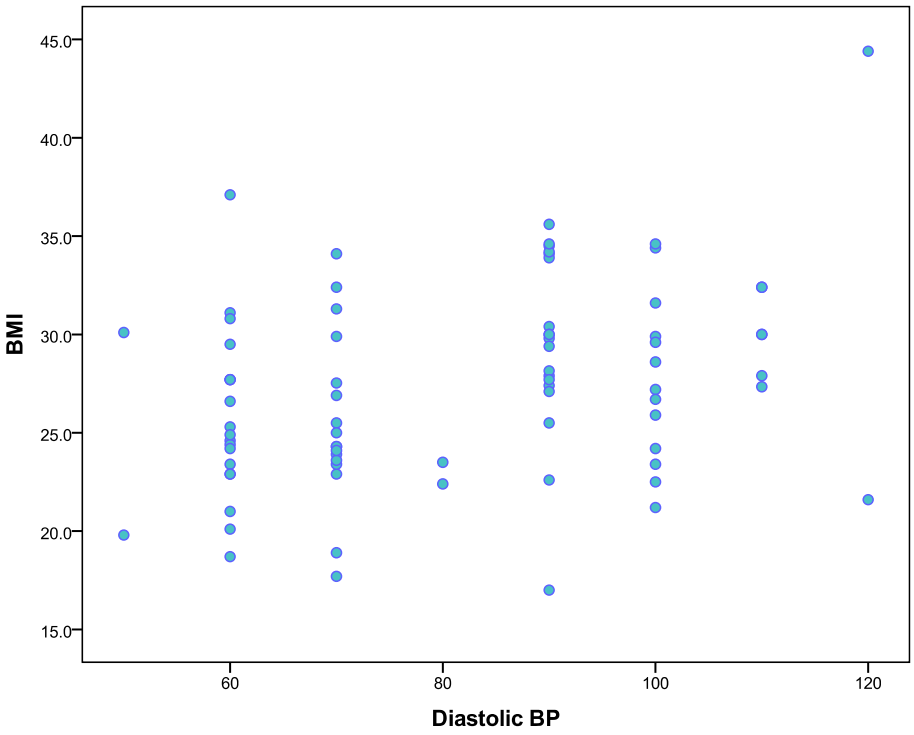
FIGURE-15:SCATTER PLOTS WITH SIGNIFICANT CORRELATIONS

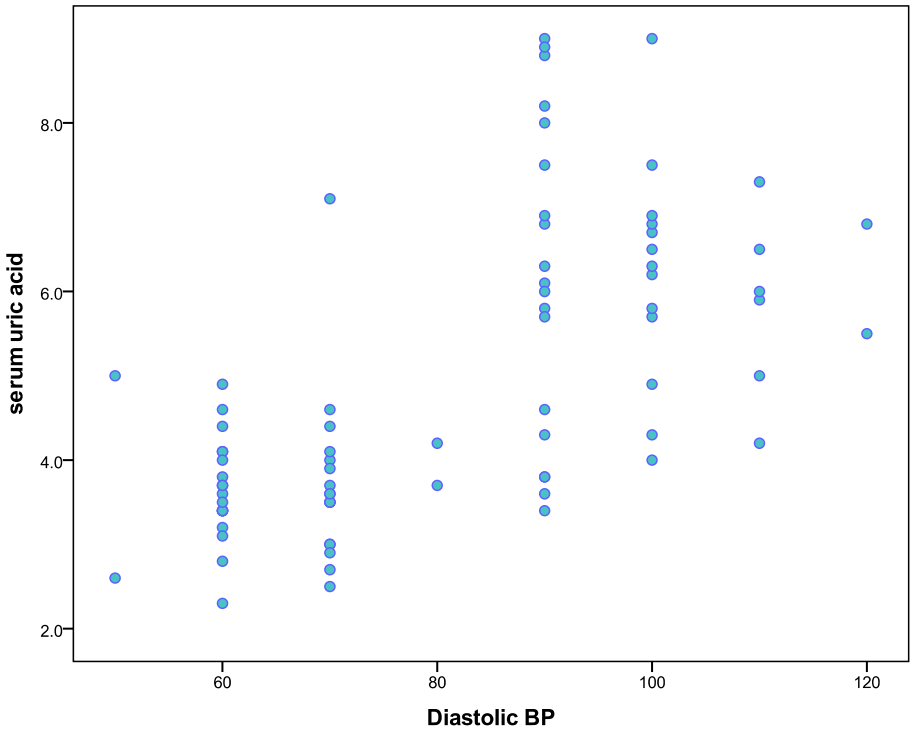


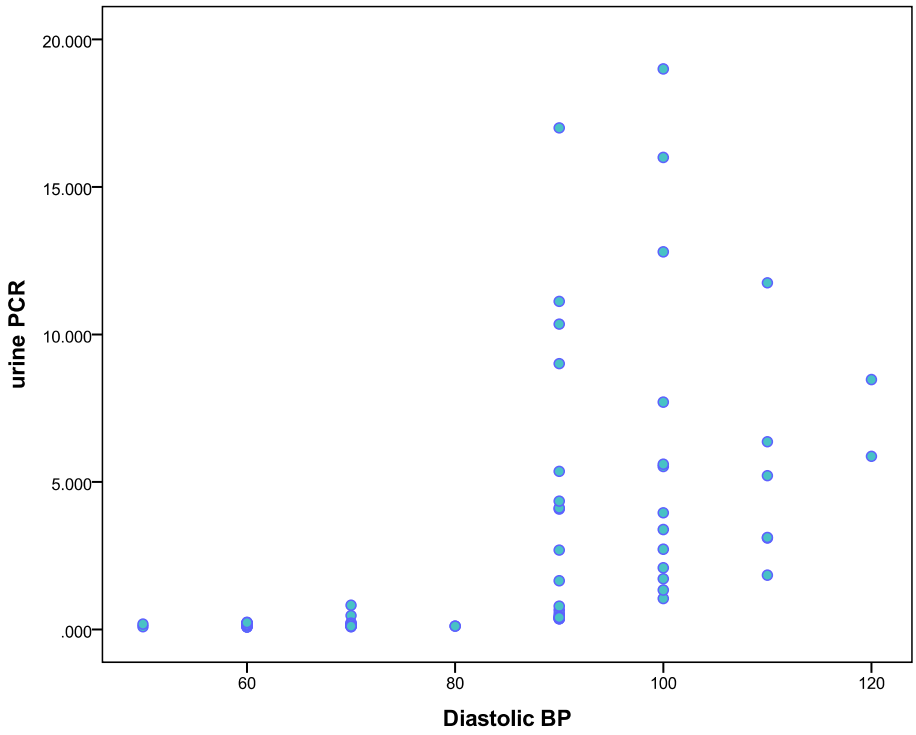


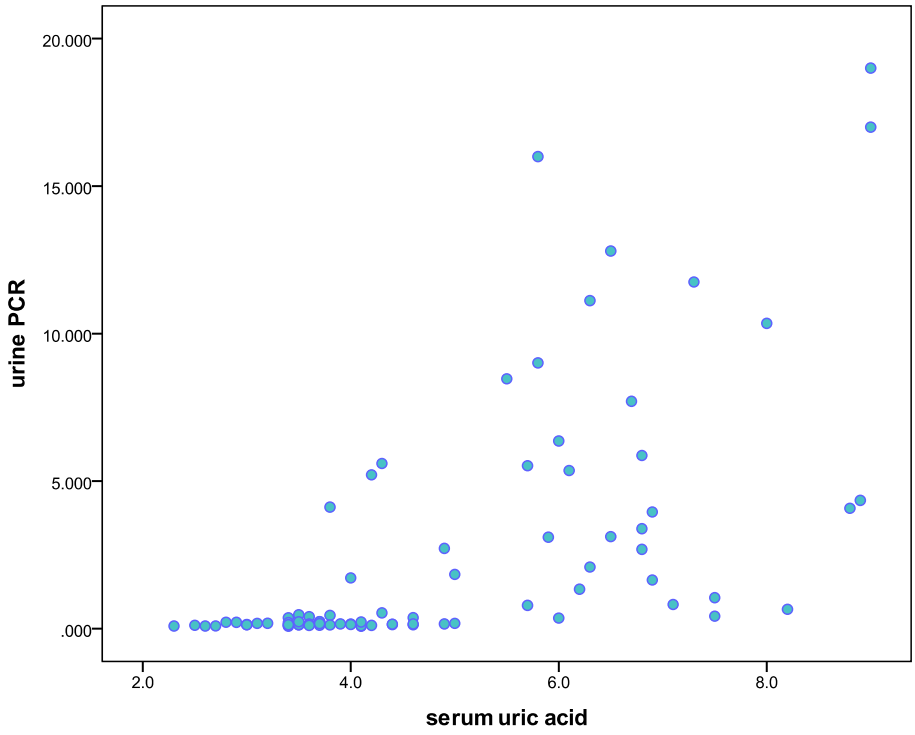

serum uric acid
8.0
6.0
4.0
2.0
80
100
120
140
160
180
200
Systolic BP


Gestational age
40
35
30
25
20
.000
5.000
10.000
15.000
20.000
urine PCR


45.0
40.0
35.0
30.0
25.0
20.0
15.0
BMI
60
80
100
120
Diastolic BP


8.0
6.0
4.0
2.0
serum uric acid
60
80
100
120
Diastolic BP


20.000
15.000
10.000
5.000
.000
urine PCR
60
80
100
120
Diastolic BP


20.000
15.000
10.000
5.000
.000
urine PCR
2.0
4.0
6.0
8.0
serum uric acid

DISCUSSION

DISCUSSION

The distribution of the maternal age and gestational age are similar in the cases and the control population and their t- values are also not significant. This shows that the two groups are well matched in these characteristics.

The two groups showed different distributions in case of parameters like systolic blood pressure, diastolic blood pressure, serum uric acid levels, urine protein creatinine ratio. The t-values are also significant in these parameters among the two groups. This is expected because the diagnosis of preeclampsia is based on the systolic blood pressure, diastolic blood pressure, serum uric acid levels, urine protein creatinine ratio and the division of the study population into two groups is based on these parameters.

When the correlation between various parameters are studied using Pearson correlation coefficient, the resulting r-values are significant for gestational age & urine PCR, BMI & urine PCR, uric acid & urine PCR, diastolic blood pressure& urine PCR,BMI & systolic blood pressure, BMI & diastolic blood pressure, diastolic blood pressure& uric acid, systolic blood pressure& uric acid. But, no significant correlation is found between serum NGAL levels and body mass index.

In a study done by Aliyu et al[36], to find out the effect of obesity on the risk of preeclampsia, they were able to demonstrate that the presence of obesity increased the risk of preeclampsia in all women in the study. Similarly, in a study done by Bodnar et

al[37],to explore the dose-dependent relation between pre-pregnancy body mass index (BMI) and the risk of preeclampsia, they have confirmed that preeclampsia risk rises through most of the BMI distribution. But in a prospective, multi-centric study done by Anderson et al[38], to find out the impact of maternal BMI on development of preeclampsia, they found out that those who are overweight or obese in early pregnancy are not more likely to have term pre-eclampsia compared with women with a normal BMI.

In consistent with the studies done by Aliyu et al, Bodnar et al, in this study, the distribution of body mass index is in the higher range in women with preeclampsia when compared with the normotensive controls. The t-test value (p=0.001) obtained by comparing the means of the two groups is also significant. This finding suggests that increased body mass index poses a threat for developing preeclampsia.

Dimitri pandinis[39] et al & Lou et al[40] have shown in their study that there is a significant correlation between serum NGAL levels and body mass index.

In the studies done by Anna et al, Simonazzi et al , Patel et al, Youssef et al to find out the correlation between serum NGAL levels and preeclampsia, the two study groups, cases and controls were matched for body mass index as elevated BMI was considered as a confounding factor. But in our study, the cases and controls are not matched for BMI.

Since NGAL is also produced by adipocytes, a positive correlation was expected between serum NGAL levels and the body mass index. But the Pearson correlation coefficient between NGAL and BMI is not significant to prove any correlation ($r=0.076$, $p=0.502$). This suggests that obesity and preeclampsia are correlated but not obesity and NGAL. So it can be said that obesity may not be a confounding factor in estimation of serum NGAL levels and the pre-pregnancy BMI rather than the BMI at the time of sampling could be considered.

In our study, serum uric acid level was also significantly higher in the preeclamptic women than in the normotensive controls as indicated by t-test value($p=0.00$).

Since estrogen is an uricosuric agent, serum uric acid levels fall by 25-35% in pregnant women[41]. Apart from estrogen, expanded plasma volume, elevated GFR also leads to decrease in uric acid levels in pregnancy[41]. In women with preeclampsia, hyperuricemia is a regular finding. Impaired renal function has been considered the causative factor for hyperuricemia in preeclampsia.

Some researchers have considered the pathophysiological role of hyperuricemia in preeclampsia, as the severity of disease symptoms increases as the uric acid level increases[41]. The pathophysiological role of hyperuricemia in preeclampsia is endorsed by in-vitro culture studies and animal models in which the pro-inflammatory effects like stimulation of smooth muscle cell proliferation, inhibition of endothelial cell proliferation, promotion of endothelial damage[41].

In the study done by Pereira et al[42] to evaluate the use of uric acid as a marker of severity of pre-eclampsia, they were able to find significant correlations between uric acid and systolic blood pressure. In a similar study done by Pramanik et al[43] to assess whether serum uric acid can be used as a biochemical indicator or not in preeclamptic patients, they were able to demonstrate a positive correlation between NGAL levels and elevated systolic pressure. Consistent with these findings, in this study, a significant positive correlation is found between serum uric acid levels and systolic BP (r=0.549,p=0.00). A linear relation is found between these two parameters by scatter plot analysis.

In the same studies done by Pereira et al & Pramanik et al, they have found a significant correlation between diastolic Blood pressure and serum uric acid levels. Similarly in this study we have found a significant positive correlation between serum uric acid level and diastolic Blood pressure(R=0.628,P=0.00). A linear relation is found between these two parameters by scatter plot analysis.

The significance of this elevated uric acid levels in preeclampsia and its correlation with the systolic and diastolic blood pressure, in the context of role of hyperuricemia in the pathogenesis of preeclampsia remains to be elucidated[44].

Nischinta et al[45] have done a study to find out the correlation between 24 hour urine protein, spot urine protein creatinine ratio and uric acid. They were able to demonstrate a positive and statistically significant correlation between serum uric acid level and urine protein creatinine ratio. Similarly in this study, a statistically

significant positive correlation is found between serum uric acid level and spot urine protein creatinine ratio(r=0.626,p=0.00). The scatter plot analysis between these two parameters also showed a linear relation.

In the study done by Anna et al[31], they have concluded that serum NGAL levels were higher in the preeclamptic women in all three trimesters when compared with the control population. Although Kim et al[47] have also demonstrated a statistically significant difference in NGAL level between the two groups, the range of NGAL levels in the two groups were similar(66.1-575.4 ng/ml in cases and 7.0-669.7ng/ml in controls).

Simonazzi et al[35] have done a cross sectional case control study, to compare the serum and urinary NGAL levels in preeclampsia and in normal pregnancies and also to find out the correlation between NGAL and blood pressure, creatinine, uric acid,24-hour proteinuria. They could not demonstrate a statistically significant difference in the NGAL levels between the two populations. They were not able to demonstrate a positive correlation between NGAL and uric acid, creatinine, blood pressure.

In a study done by Dogan et al[46], to evaluate the serum NGAL levels and plasma Nitric oxide in preeclamptic women and normal controls, the investigators were not able to find a statistically significant difference in the NGAL levels between the two groups. But they were able to find a statistically significant correlation between NGAL levels and BMI.

In our study, the serum NGAL ranged from 40-900 ng/ml in cases and from 110-795 ng/ml in controls. The distribution range of NGAL in cases covers the distribution range of controls. This shows that there is no difference in NGAL between cases and control.

The correlation coefficients between the NGAL levels and other parameters like maternal age, gestational age, systolic blood pressure, diastolic blood pressure, uric acid levels, urine PCR are also not statistically significant. These findings of this study are consistent with those of Simonazi et al[35] and Dogen et al[46] and contrast from the findings of Anna et al[31], Kim et al[47].

CONCLUSION

CONCLUSION

1. Serum NGAL levels are not significantly elevated in patients with preeclampsia when compared with the normotensive controls.

2. There is no significant correlation between serum NGAL levels and systolic blood pressure, diastolic blood pressure, serum uric acid, urine PCR, maternal age and gestational age.

SUMMARY

SUMMARY

Preeclampsia is one of the leading causes of maternal and neonatal morbidity and mortality in pregnant women. The diagnosis of preeclampsia is made based on the measurement of blood pressure, urinary proteins after 20 weeks of gestation. By the time the diagnosis is made and the treatment is commenced, the damaged concurred to the mother and fetus becomes irreparable. The exact etiopathogenetic mechanisms for the development of preeclampsia is not known.

There is a wide spread endothelial activation and damage in preeclampsia. Almost all of the organ systems are involved in the endothelial damage. Kidneys being highly vascular, becomes one of the common and severely injured organs. Neutrophil Gelatinase Associated Lipocalin (NGAL) has been proved as a kidney injury marker. So, in this study we have compared the serum NGAL levels in patients with preeclampsia and gestational age matched controls using NGAL ELISA kit.

There was no significant difference in the serum NGAL levels between the two groups and there was no significant correlation between serum NGAL levels and the other parameters like systolic BP, diastolic BP, serum uric acid and urine protein creatinine ratio. So, serum NGAL levels cannot be used as a diagnostic marker of preeclampsia.

FUTURE SCOPE

FUTURE SCOPE OF THE STUDY

1. Association between preeclampsia and other protein markers like Kidney Injury Molecule-1,Liver Fatty Acid Binding Protein, N-Acetyl Glucose aminidase, endoglin, soluble Fms like tyrosine kinase-1 could be studied.

2. Apart from these protein markers, other small molecules involved in the pathogenesis of preeclampsia could be studied.

3. The entire spectrum of metabolites in preeclampsia could be studied by techniques like liquid/gas chromatography coupled with tandem mass spectrometry.

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ANNEXURE

DATA COLLECTION TOOL(QUESTIONNAIRE)

1. Serial number:
2. Age:
3. Socioeconomic status:
4. Height:
5. Weight:
6. Blood pressure:
7. LMP:EDD:
8. Gestational age in weeks:
9. Patient's blood group:
10. Husband's blood group:
11. Child's blood group:
12. Family history of hypertension:
13. Dietary history:
14. History of illness:

Diabetes: Yes/No Hypertension: Yes/No

Any other medical illness: Yes/No

15. Lab investigations:

    Urine protein Creatinine ratio

    Uric acid